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The hepatoprotective effect of gooseberry and black mulberry extracts against carbon tetrachloride-induced liver injury in rats

Shimaa Elmasry[1*] and Mahmoud Moawad[2]

Abstract

Background: Liver is the vital organ of the human body responsible for nutrition, immunity, and metabolism. Carbon tetrachloride (CCl_4) is an environmental pollutant that causes hepatotoxicity. This study aimed to evaluate the possible hepatoprotective effect of aqueous and ethanolic extracts of gooseberry and black mulberry on liver injury induced by CCl_4 in rats.

Results: CCl_4 caused significant ($P \leq 0.05$) elevation in the liver function tests and hydroxyproline (a major marker of fibrosis); also, there was a significant increase in the hepatic malondialdehyde (MDA), nitric oxide (NO), and plasma inflammatory biomarkers, whereas a significant decrease in the hepatic reduced glutathione (GSH), glutathione peroxidase (GPx), and plasma adiponectin levels was observed in the CCl_4-treated group compared with control. These results were also confirmed by histological examination of liver tissue. Administration of gooseberry or black mulberry extracts alone decreased the hepatic level of hydroxyproline, ameliorated the antioxidant/oxidant status in liver tissue, and decreased the pro-inflammatory cytokines compared to normal control. Treatment with the tested extracts along with CCl_4 was effectively able to ameliorate the abovementioned imbalances induced by CCl_4 and protect the liver tissue.

Conclusion: These results indicate that gooseberry and black mulberry extracts have a hepatoprotective effect against carbon tetrachloride-induced liver injury in rats.

Keywords: Carbon tetrachloride, Liver injury, Liver fibrosis, Gooseberry, Black mulberry, *Physalis peruviana* L., *Morus nigra*

Background

Liver is the largest gland in the body and the key organ of immunity, nutrition, and metabolism (Ahmed, Eldemardash, & Ali, 2019). Hepatic damage caused by chemicals or infectious agents is associated with a disturbance of the metabolic functions of liver and may lead to progressive liver fibrosis, cirrhosis, and liver failure (Cullen, 2005). *Carbon tetrachloride (CCl_4)* is a common toxin that is widely used for experimental induction of liver injury and fibrosis

(Weiler-Normann, Herkel, & Lohse, 2007). The principal causes of CCl_4-induced hepatic injury and fibrosis are increased lipid peroxidation, generation of free radicals, and the depletion of antioxidant status (Shahidi & Zhong, 2010). Accordingly, antioxidant supplementation can reduce the risk of liver injuries and offer insights into delaying or preventing the occurrence and development of hepatic fibrosis (Deng et al., 2012). Rajesh and Latha (2004) indicated that various herbal extracts may protect liver against CCl_4-induced oxidative stress by reverse the increased levels of lipid peroxidation and enhancing the decreased activities of antioxidant enzymes. Cape gooseberry (*Physalis peruviana* L.) is belonging to the

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family *Solanaceae* and genus *Physalis*, it is known locally in Egypt as Harankash and in English-speaking countries as goldenberry, and it has many medicinal and edible uses as a promising fruit (Ramadan, 2011). The fruits are succulent golden spheres the sizes of marbles, each fruit contains about 100–200 small seeds (Luis, Claudia, Eduardo, & Misael, 2011). Gooseberry is a widely used herb in folk medicine for several diseases. It was reported that *Physalis peruviana* L. have various bioactive compounds, some of which have a strong antioxidant property and prevent peroxidative damage of liver cells (Al-Olayan et al., 2014). Wu et al. (2006) illustrated that the ethanolic extract of gooseberry fruit had higher antioxidant properties than the aqueous extract. The antioxidant properties associated with the fruits are due to their high levels of polyphenols, vitamins A (α-carotene, β-carotene, and β cryptoxanthin), and vitamin C. The specific active constituents of *Physalis peruviana* L. are Physalins A, B, D, F, and glycosides, which show anticancer activity (Wu et al., 2004). *Physalis peruviana* L. contains *also* 28-hydroxywithanolide, withanolides, phygrine, kaempferol, quercetin diglycosides, and triglycosides (Arun & Asha, 2007). Some of these active components were previously reported to have antitumor, cytotoxic inhibition of the ubiquitin-proteasome pathway (Ausseil et al., 2007); immunomodulatory (Soares et al., 2006), anti-inflammatory, and antiallergic activities (Lee, Pan, Chen, & Chen, 2008). *Black mulberries* (*Morus nigra*) are belonging to the family Moraceae. Its flavonoids have strong antioxidant activity. The fruits are used in medicine against inflammation and to stop bleeding, the bark is used for toothache, and the leaves for treating snakebites and used as an antidote to poison (Feng et al., 2015). *M. nigra* is rich in essential fatty acids as linoleic acid, polyphenols, flavonoids, and anthocyanins, which are accountable for its color (Ross & Kasum, 2002; Salcedo, Sendra, Barrachina, Martínez, & Hernández, 2016). Anthocyanins extracted from mulberry has been reported to present neuroprotective (Kang et al., 2006) and antitumor activity (Huang, Shih, Chang, Hung, & Wang, 2008). It has been also shown that *M. nigra* has a protective action against oxidative damage to membranes and biomolecules (Memon, Memon, Luthria, Bhanger, & Pitafi, 2010). The flavonoids compound of black mulberry has been shown to have hepatoprotective activity as previously reported (Mallhi, Qadir, Khan, & Ali, 2014).

Hence, the present study was aimed to investigate the possible hepatoprotective effects of gooseberry and black mulberry (water and ethanolic extracts) against CCl_4-induced hepatic injury and fibrosis in male albino rats.

Methods

Chemicals and plant materials

Carbon tetrachloride (CCl_4) was purchased from Sigma for chemicals company, Cairo. Egypt. The mature and fresh fruits of gooseberry and black mulberry were purchased from the local markets of Cairo, Egypt. The fruits were cleaned, oven-dried at temperature 50℃, then were ground in a mortar. For the preparation of water and ethanolic extracts, 250 g of dried plant fruits were mixed with 1000-mL solvents [water and ethanol (95%)] in dark-colored bottles and extracted at room temperature overnight. The resulted suspension was filtered through Whatman filter paper and the operation was repeated 2 times for re-extraction, then the solvent was removed by using a rotary evaporator at 40 ℃. The extracts were kept at − 8 ℃ until use.

Measurement of total flavonoids, total polyphenols, and total antioxidants activity

Total flavonoids in water and ethanolic extracts of gooseberry and black mulberry were assessed according to Sakanaka, Tachibana, and Okada (2005). Flavonoids were determined as quercetin from a calibration curve (Merfort et al., 1997) and expressed as mg/g of the extract. Total phenolic content was determined as described by Kähkönen et al. (1999), using the Folin-Ciocalteu procedure and expressed as mg gallic acid equivalents (GAEs)/g of the extract. The free radical scavenging activity (total antioxidant activity) of extracts was measured by α,α-diphenyl-β- picryl- hydrazyl (DPPH) according to Brand-Williams, Cuvelier, and Berset (1995). Radical scavenging activity was expressed as a percentage of inhibition/g of the extract and was calculated using the formula: % Radical scavenging Activity = [(control OD − sample OD)/control OD] × 100.

Laboratory animals

Adult male Sprague-Dawley rats weighing 150–160g were used for this study, and all were kept in the animal house of the Medical Research Center, Faculty of Medicine, Ain Shams University, Cairo, Egypt. The animals were housed in stainless steel cages and maintained under standard laboratory conditions (temperature 25 ± 5℃) with dark and light cycle (12/12h). The acclimatization period lasted for 7 days. All animals were fed with a standardized pellet diet and water ad libitum throughout the experimental period (5 weeks). The protocol of this study was approved by the research ethical approval committee of the Medical Research Center, Ain Shams University.

Experimental design

The concentrated extracts were dissolved in distilled water immediately before use and administered to rats

in a dose of 250 mg/kg b.wt according to Taj, Khan, Sultana, Ara, and Ehteshamul-Haque (2014).

Sixty rats were randomly divided into 10 groups of 6 animals each. *Group 1* (control): orally received 0.5ml corn oil/kg b.wt, 3 times a week. *Group 2* (CCl_4): the animals were orally given CCl_4 (1 ml/kg body weight CCl_4/corn oil, 1:1 v/v), 3 times a week. *Group 3* (GW): were given orally water extract of gooseberry (250 mg/kg b.wt) daily. *Group 4* (GE): were orally given ethanolic extract of gooseberry (250 mg/kg b.wt) daily. *Group 5* (MW): were orally given water extract of black mulberry (250 mg/kg b.wt) daily. *Group 6* (ME): were orally given ethanolic extract of black mulberry (250 mg/kg b.wt) daily. *Groups 7, 8, 9, and 10* were orally given CCl_4 (1 ml/kg body weight CCl_4/corn oil, 1:1 v/v), 3 times a week, and daily received 250 mg/kg b.wt of GW, GE, MW, and ME extracts, respectively. The animals were observed daily for mortality and the body weight of all rats was recorded weekly.

At the end of the 5th week, all rats were sacrificed under ether anesthesia. Blood samples were collected from the hepatic portal vein into heparinized tubes and centrifuged at 3000 rpm for 30 min at 4°C; plasma samples were stored at – 20 °C for further assessment. The liver tissues were immediately removed, washed in cold saline, dried on filter paper, and weighed using an electronic balance. Parts of the liver tissue were immediately fixed in 10% neutral phosphate-buffered formalin for histopathological study. Other parts were used for tissue homogenate preparation, in which, liver tissue was homogenized in 5 ml cold buffer (i.e., 50 mM potassium phosphate, pH 7.5. 1 mM EDTA) per gram tissue. The homogenate was centrifuged at 5000 rpm for 20 min; the supernatant was removed and kept at –80°C till processed.

Biochemical assays

Plasma alanine transaminase (ALT) and aspartate transaminase (AST) activities were determined colorimetrically according to Murray (1984); plasma alkaline phosphatase (ALP) activity was measured by colorimetric method kit as described by Belfield and Goldberg (1971). Plasma alpha-fetoprotein (AFP) level was estimated by the immune-enzymatic colorimetric method according to Acosta (1983). Hepatic hydroxyproline was measured using commercial diagnostic kit K555-100 (BioVision Research Products, CA, USA). Reduced glutathione (GSH) was determined colorimetrically according to Beutler, Duron, and Kelly (1963). Glutathione peroxidase (GPx) was assessed by UV method kit as described by Paglia and Valentine (1967). Malondialdehyde (MDA) and nitric oxide (NO) were determined by the colorimetric method kits according to Montgomery and Dymock (1961) and Ohkawa, Ohishi, and Yagi (1979)

respectively. Plasma interferon-gamma (IFN γ) was assessed using Rat IFN-gamma ELISA Kit, RayBiotech, USA. Tumor necrosis factor-alpha (TNF-α), nuclear Factor κB (NF-κB), and adiponectin were determined using Rat ELISA kit, MyBioSource, USA.

Histopathological examination of liver lesions

Liver specimens were fixed in 10% formalin solution and processed routinely for paraffin embedding. Sections (4-μm-thick) were deparaffinized, stained with hematoxylin and eosin solutions (H&E), and examined under the light microscope (Bancroft, Stevens, & Turner, 1996).

Statistical analysis

The data were analyzed using SPSS 26 for windows. All variables were compared using one-way analysis of variance (ANOVA) followed by LSD post hoc test. $P \leq 0.05$ was considered statistically significant (Levesque, 2007).

Results

The data presented in Table 1 illustrate the values of total flavonoids as mg quercetin equivalent (QE)/g of the extract and total phenols as mg gallic acid equivalents (GAEs)/g of the extract, as well as total antioxidant capacity indicated by the 1,1-diphenyl-2-picryl hydrazyl DPPH free radical scavenging activity. Results showed that the GW extract has the highest level of total flavonoids. Concerning total phenols, the highest level was found in the MW extract. The ethanolic extracts of gooseberry (GE) have more antioxidant capacity than other extracts.

As shown in Figs. 1, 2, and 3, administration of CCl_4 caused a significant ($P < 0.05$) decrease in the body weight and increase in liver weight and relative liver weight (hepatosomic index) of rats. Gooseberry and black mulberry extract markedly reduced the liver weight and hepatosomic index in treated groups when compared with the CCl_4 group.

The results presented in Table 2 indicated that rats treated with CCl_4 have significantly ($P < 0.05$) elevated levels of plasma ALT, AST, ALP, and AFP compared to the normal control group. Likewise, the collagen deposition as evidenced by hepatic hydroxyproline in CCl_4 treated rats was significantly higher than the normal level of a control group. The level of liver enzymes in groups that were administered with the tested extracts alone still not different from the control group although they showed a marked decrease in the liver hydroxyproline level. However, treatment with gooseberry and black mulberry extracts along with CCl_4 significantly reduced the elevated levels of ALT, AST, and ALP (the most reduced level was found in the CCl_4+ ME group), alfa fetoprotein, and hydroxyproline (lowest level was found in CCl_4+ GE group) compared to the hepatotoxin group.

Table 1 Total flavonoids, phenols, and antioxidant capacity of gooseberry and black-mulberry extracts

Extract type	Total flavonoids (mg as QE/1 g of extract)		Total phenols (mg as GAEs/1 g of extract)		Total antioxidants capacity (% of inhibition/1 g of extract)	
	Gooseberry	Black-mulberry	Gooseberry	Black-mulberry	Gooseberry	Black-mulberry
Water extract	34.13	27.22	29.11	32.11	78.92	76.08
Ethanol extract	30.06	16.62	25.31	31.22	80.01	75.32

A significant decline ($P < 0.05$) was observed in the hepatic GSH level and GPx enzyme activity after CCl_4 treatment compared to normal control. In contrast, a marked increase in MDA and NO was found in the liver of CCl_4-treated animals. Interestingly, the oxidant/antioxidants status was improved with the treatment of healthy rats by gooseberry and black mulberry extracts compared to normal control rats (GE group showed the most ameliorated effect). Administration of gooseberry and black mulberry extracts along with CCl_4 resulted in a significant increase in hepatic GSH and GPx (highest level was found in GW group), and reduction in MDA and NO (lowest level was found in GE group) comparable to the hepatotoxic group (Table 3).

As illustrated in Table 4, high levels of pro-inflammatory cytokines (IFN γ and TNF-α) and the transcription factor nuclear factor-κB (NF-κB) were detected in the plasma of rats after CCl_4 treatment. Also, CCl_4 caused a significant decline in serum concentration of the anti-inflammatory adipocyte-derived adiponectin comparing to control rats. Treatment of rats with the tested extracts alone significantly decreased the levels of pro-inflammatory cytokines when compared to the control group. The elevated levels of IFN γ, TNF-α, and NF-κB induced by CCl_4 were significantly ($P < 0.05$) alleviated, while the decreased

level of adiponectin increased markedly after treatment with gooseberry and black mulberry extracts.

As indicated by H&E staining of liver tissue (Fig. 4), samples from the normal control group and treated groups (GW, GE, MW, and ME) showing normal architecture with the presence of central vein surrounded by hepatocytes which separated by blood sinusoids, the hepatocytes are polyhedral shape with a central rounded nucleus, respectively. On the other hand, significant destruction of hepatic architecture, apparent portal fibrosis, activation of hepatic stellate cells as well as periportal aggregations of inflammatory cells, and congestion of blood vessels were identified in the liver tissue of rats after 5 weeks of CCl_4 administration. The liver tissue of CCl_4-intoxicated rats that were treated with GW showed improvement in the morphology of liver comparing to the CCl_4 group with few lymphocyte infiltrations and less periportal fibrosis. CCl_4 +GE group showed the most improvement in hepatic architecture near to control group with few lymphocyte infiltrations and no fibrosis. Amelioration of hepatocytes with some fibrosis and few lymphatic infiltrations around blood vessels was shown in the liver tissue from the CCl_4+MW group, while treatment with ME along with CCl_4 cause improvement in the morphology of liver tissue with few lymphocyte infiltrations and less periportal
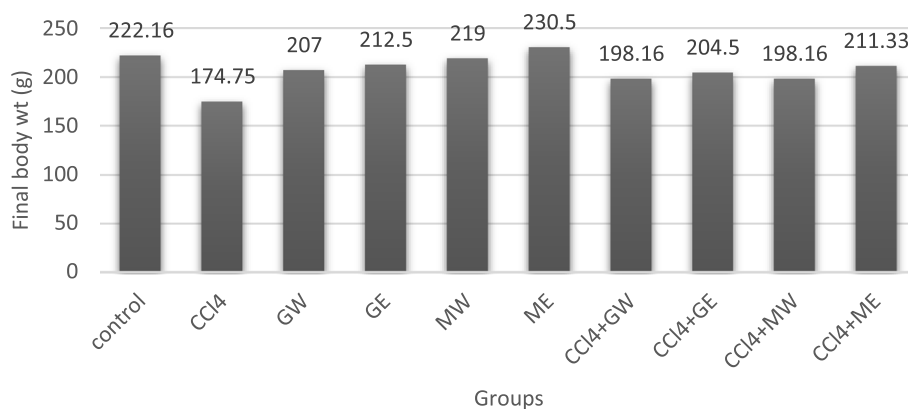
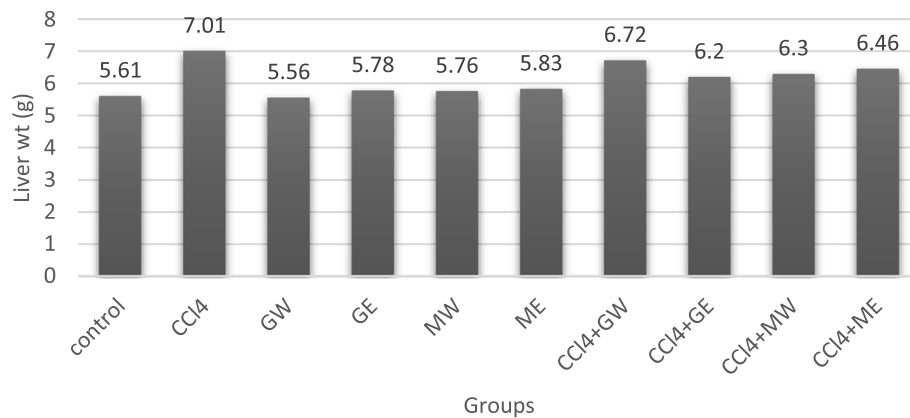**Fig. 1** Final body weight (g). Values are expressed as means ± SE, $n= 6$ ($P \leq 0.05$)

Fig. 2 Liver weight (g). Values are expressed as means ± SE, *n*= 6 (*P*≤0.05)

fibrosis in comparison with CCl_4 group. The histo-pathological findings are summarized in Table 5.

Discussion

Since ancient times, natural plant products have been used as a remedy and protection from various diseases. Therefore, we sought to investigate the effect of aqueous and ethanolic extracts of gooseberry and black-mulberry as a possible hepatoprotective agent in the CCl_4-induced hepatic injury. Our results indicated that administration of aqueous and ethanolic extracts of both berries at a daily dose of 250 mg/kg b.wt for 5 weeks induced hepatic protection against CCl_4 and was associated with reduced the biomarkers of liver injury and oxidative stress and reduced cytokine content associated with inflammation and ameliorated liver architecture. It is clear from Table 1 that GW, GE, MW, and ME possesses potent total antioxidant activity and contain reasonable amounts of total flavonoids and phenols, with GW and GE have the highest level of antioxidant capacity and total flavonoids, while MW and ME have the highest

level of total phenols. This could be attributed to the antioxidant activity of these natural extracts. Previous studies also have demonstrated that gooseberry and black mulberry extracts exhibit many bioactive features including antioxidant and anti-inflammatory properties due to the presence of large amounts of phenolic compounds and flavonoids. The qualitative analysis of *Physalis peruviana* L. ethanolic extract performed by Sathyadevi and Subramanian (2015) revealed the presence of biologically active compounds such as phenols, flavonoids, glycosides, sterols, saponins, tannins, lactones, and alkaloids. The fruit extract was also found to contain significant amounts of rutin, myricetin, quercetin, and kaempferol. Janbaz, Saeed, and Gilani (2004) suggested that the antioxidant and antifibrotic effect of a gooseberry may be due to the presence of a high amount of quercetin which has strong antioxidant activity and anti-inflammatory properties (Boots et al., 2008). In addition to quercetin, gooseberry also contains kaempferol 3-Orutinoside that considered as antioxidant due to its ability to scavenge free radicals and ROS (Tatsimo,
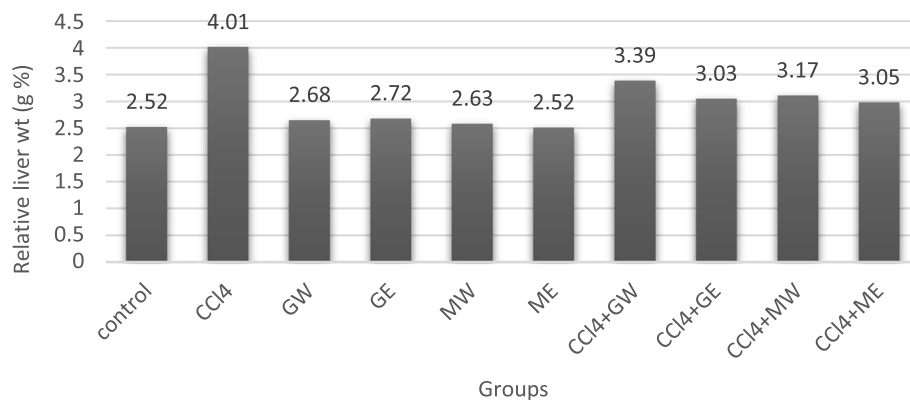

Fig. 3 Relative liver weight. Values are expressed as means ± SE, *n*= 6 (*P*≤0.05)

Table 2 Biomarkers of liver lesions

Groups	ALT (U/l)	AST (U/l)	ALP (U/l)	AFP (ng/ml)	Hydroxy proline (mg/g tissue)
Control	38.41±2.25[a, c]	103.20 ± 2.03[a]	119.08±0.60[a]	0.566±0.01[a]	28.02±0.54[a]
CCl$_4$	224.56± 2.59[b]	333.34 ± 2.11[b]	201.83±2.08[b]	4.350±0.40[b]	59.33±0.94[b]
GW	37.04±0.90[a, c]	104.25 ± 0.44[a]	117.39±0.70[a, c]	0.582±0.02[a]	22.66±0.28[c, d]
GE	39.71 ±0.84[c]	106.70 ± 2.37[a]	117.48±1.11[a, c]	0.661±0.04[a]	21.48±0.33[d]
MW	34.71 ± 0.36[a]	105.83 ± 1.42[a]	118.33±0.57[a, c]	0.653±0.03[a]	23.98±1.23[c]
ME	35.59 ± 0.14[a, c]	104.58 ± 1.40[a]	115.20±1.66[c]	0.650±0.01[a]	22.40±0.93[c, d]
CCl$_4$+GW	66.38 ± 1.64[d]	128.75±2.13[d]	139.01±0.46[d]	1.75±0.06[c]	37.01±0.60[e]
CCl$_4$+GE	88.16±1.30[e]	134.66±1.56[e]	142.00±0.78[d]	1.65±0.04[c]	33.13±1.46[f]
CCl$_4$+MW	89.02±2.10[e]	134.98±1.81[e]	138.78±0.25[d]	1.95±0.05[d]	37.04±0.57[e]
CCl$_4$+ME	64.62±1.69[d]	120.60±0.68[f]	138.10±1.70[d]	2.01±0.04[d]	37.11±1.04[e]

Values are expressed as means ± SE, *n*= 6. There was no significant difference between means have the same letter in the same column (*P*≤0.05)

Tamokou, Havyarimana, et al., 2012). Moreover, the study of Ahmed (2014) on the ethanolic extract of *Physalis peruviana* L. detected the presence of phytosterols and saponin also which have antioxidant activity. Furthermore, *Physalis peruviana* L. extracts contain many withanolide glycosides which are natural steroidal lactones produced by plants in the Solanaceae and often have many health benefits such as anti-inflammatory activity (Lan, Chang, Pan, et al., 2009). It has been also reported that the water extract of *Physalis peruviana* contains phytochemicals, flavonoids, saponins, and phenols (Arun & Asha, 2007).

Bao et al. (2016) showed that black mulberry extract contains large amounts of flavonoids, phenolic compounds, and anthocyanins, which exhibit many bioactive features including antioxidant, anti-inflammatory, anti-obesity, anti-atherogenesis, and anticancer properties. It has been reported that black mulberry is a plant rich in fatty acids, such as linoleic, stearic, and oleic acids and other molecules that possess antioxidant, anti-inflammatory, and analgesic effects (Feng et al., 2015).

The results of a qualitative investigation performed by Mallhi et al. (2014) revealed the presence of luteolin, quercetin, and isorhamnetin in aqueous methanolic extract of *M. nigra.* Luteolin (Domitrović, Jakovac, Milin, & Radošević-Stašić, 2009), isorhamnetin (Kim, Kim, Choi, & Lee, 2012), and quercetin (Janbaz et al., 2004) all are known for their antioxidant and hepatoprotective potential. The results presented by Chang et al. (2011) illustrate that ethanolic extract of mulberry exhibited radical scavenging and reducing activity due to its high content of phenolic compounds such as maclurin, rutin, isoquercitrin, and resveratrol.

Carbon tetrachloride is a common environmental pollutant that causes hepatocyte injuries and altered membrane integrity, and as a result, enzymes in hepatocytes leak out (Hu et al., 2000). CCl$_4$ is metabolized by the cytochrome P450 system to highly reactive trichloromethyl radical (CCl$_3^\bullet$) and trichloromethyl peroxyl radical (CCl$_3$OO$^\bullet$), which cause liver cell destruction. These free radicals initiate autoxidation of cytoplasmic membrane fatty acids and cause accumulation of lipid-

Table 3 Oxidant/antioxidant status

Groups	GSH (mmol/g tissue)	GPx (U/g tissue)	MDA (nmol/g tissue)	NO (µmol/g tissue)
Control	50.86±2.35[a]	119.30 ± 0.66[a]	49.90±0.40[a]	3.68±0.09[a]
CCl$_4$	21.80± 0.35[b]	69.30 ± 0.47[b]	96.45±2.87[b]	7.40±0.11[b]
GW	56.35±1.49[c]	122.86 ± 1.11[a, c]	45.16±0.80[c]	2.53±0.67[c]
GE	62.27 ± 1.41[d]	124.58 ± 1.79[c, d]	40.39±0.21[d]	2.23±0.07[c]
MW	57.80 ± 1.32[c]	122.13 ± 1.94[a, d]	43.75±0.84[c, d]	2.77±0.18[c]
ME	56.43 ± 1.42[c]	123.54 ± 1.15[a, d]	43.51±1.23[c, d]	2.70±0.04[c]
CCl$_4$+GW	40.66 ± 0.42[e]	98.66±1.18[e]	63.53±0.44[e]	4.47±0.23[d]
CCl$_4$+GE	35.26±1.13[f]	98.61±3.18[e]	55.67±2.75[f]	4.10±0.09[a, d]
CCl$_4$+MW	34.02±1.18[f]	87.92±0.77[f]	74.82±0.96[g]	5.40±0.48[e]
CCl$_4$+ME	36.63±1.51[f]	96.21±2.99[e]	68.58±1.07[h]	4.35±0.30[d]

Values are expressed as means ± SE, *n*= 6, There was no significant difference between means have the same letter in the same column (*P*≤0.05)

Table 4 Plasma cytokines levels

Groups	IFN γ (pg/ml)	TNF-α (pg/ml)	NF-KB (ng/ml)	Adiponectin (pg/ml)
Control	44.74±1.05[a]	32.21±0.96[a]	94.71 ± 1.17[a]	9.55±0.17[a]
CCl$_4$	87.72± 0.62[b]	75.90±0.64[b]	138.48 ± 2.73[b]	4.01±0.20[b]
GW	36.21±0.38[c]	25.76±0.60[c]	83.45 ± 0.64[c]	8.16±0.15[c]
GE	34.72 ±0.70[c]	21.90±0.36[d]	74.08 ± 1.60[d]	7.91±0.10[c]
MW	35.71 ± 1.14[c]	25.66±0.71[c]	86.81 ± 0.89[c]	8.30±0.29[c]
ME	34.04 ± 1.00[c]	26.55±0.38[c]	83.52 ± 0.42[c]	8.00±0.18[c]
CCl$_4$+GW	52.30 ± 0.78[d]	55.48±1.22[e]	105.21±3.04[e, f]	6.15±0.22[d, e]
CCl$_4$+GE	52.95±1.50[d]	49.24±0.22[f]	106.99±0.44[e]	7.06±0.27[f, g]
CCl$_4$+MW	57.00±2.21[e]	55.51±1.29[e]	110.68±3.92[e]	6.55±0.26[e, g]
CCl$_4$+ME	53.15±2.77[d]	54.39±1.26[e]	99.83±2.66[a, f]	6.81±0.27[g]

Values are expressed as means ± SE, n= 6, There was no significant difference between means have the same letter in the same column ($P \leq 0.05$)

derived oxidation products, thiobarbituric acid reactive substances (TBARS), leading to cell membrane damage and eventually liver diseases (Jaeschke, 2011). The body's own antioxidants system (endogenous antioxidant) or supplemented antioxidant from the diet (exogenous antioxidant) can counteract oxidative stress induced by CCl$_4$ by neutralizing the excess free radicals and protect cells against their toxic effects (Wahid, Hamed, Eltahir, & Abouzied, 2016).

According to biochemical analysis and histopathological examination of liver tissue, administration of CCl$_4$ at a dose of 1 ml/kg body weight 3 times a week successfully induced hepatoxicity. In this study, all groups showed no mortality, which was in accordance with Muriel, Moreno, Hernández Mdel, Chávez, and Alcantar (2015) who reported that after 1 month of chronic CCl$_4$ intoxication 100% of rats survived. However, mortality appeared only after 2 months of intoxication (5.55%), increased to 26.6% by 3 months, and reached 55.5% at month 4 of chronic intoxication with CCl$_4$.

Hepatosomatic index (relative liver weight to body weight) is fundamental to liver injury diagnosis (Saxena, Rathor, Mahour, Saxena, & Bajaj, 2010). The results presented in Figs. 1, 2, and 3 show significant lower body weight, higher liver weight, and relative liver weight in CCl$_4$-treated rats when compared to control. Dutta et al. (2018) showed that the increase in liver weight may be probably due to the formation of reactive oxygen species (ROS) which led to hepatic damage and liver inflammation. On the other hand, administration of aqueous and ethanolic extracts of gooseberry and black mulberry appeared to enhance the liver-body weight ratio (HSI).

In the current work, administration of CCl$_4$ markedly increased the plasma level of liver enzymes (ALT, AST, and ALP), which are indices of liver cell damage and leakage of enzymes from cells. Essawy, Abdel-Moneim, Khayyat, and Elzergy (2012) reported that the rise in

ALT activity is almost always due to hepatocellular damage and is usually accompanied by a rise in AST and ALP. Alpha-fetoprotein (AFP) is an α1-globulin secreted by fetal hepatocytes and in a small amount by other cells of the fetal gastrointestinal tract (El Raziky et al., 2013). The present data showed increased AFP in rats treated with CCl$_4$; this increase may be due to the hepatotoxic effect associated with CCl$_4$. Our results showed amelioration in plasma levels of ALT, AST, ALP, and AFP in rats treated with the tested extracts (Table 2) which is a clear indication of the improvement in the functional status of the liver and protection of cellular architecture.

Liver tissue fibrosis of the CCl$_4$ group is evident from the increased hepatic level of hydroxyproline and histopathological examination of liver tissue. Hydroxyproline is an abundant amino acid present in collagen; the presence of hydroxyproline in the extracellular matrix produced by activated hepatic stellate cells preserves the integrity and function of liver cells. Its level in liver tissues could correctly indicate the rates and progression of liver fibrogenesis (Gabr, Alghadir, Sherif, & Ghfar, 2016). Treatment with the tested extracts either alone or along with CCl$_4$ markedly reduced the hepatic collagen deposition marker, hydroxyproline, indicating their protective effects against collagen deposition and liver fibrosis (Table 2).

In this work, CCl$_4$ causes noticeable toxicity by enhancing liver lipid peroxidation (LPO), as indicated by the elevated concentration of hepatic MDA, and increased production of reactive nitrogen species (RNS) such as NO. Nitric oxide is known to react with superoxide anion, forming highly reactive peroxynitrite (ONOO–·) radical, which can cause cytotoxicity and DNA damage through LPO (Tipoe et al., 2010). It has been suggested that lipid peroxidation may be a link between tissue injury and liver fibrosis by modulating collagen gene expression (Parola & Robino, 2001). The liver intoxication induced by CCl$_4$ also causes significant depletion of the
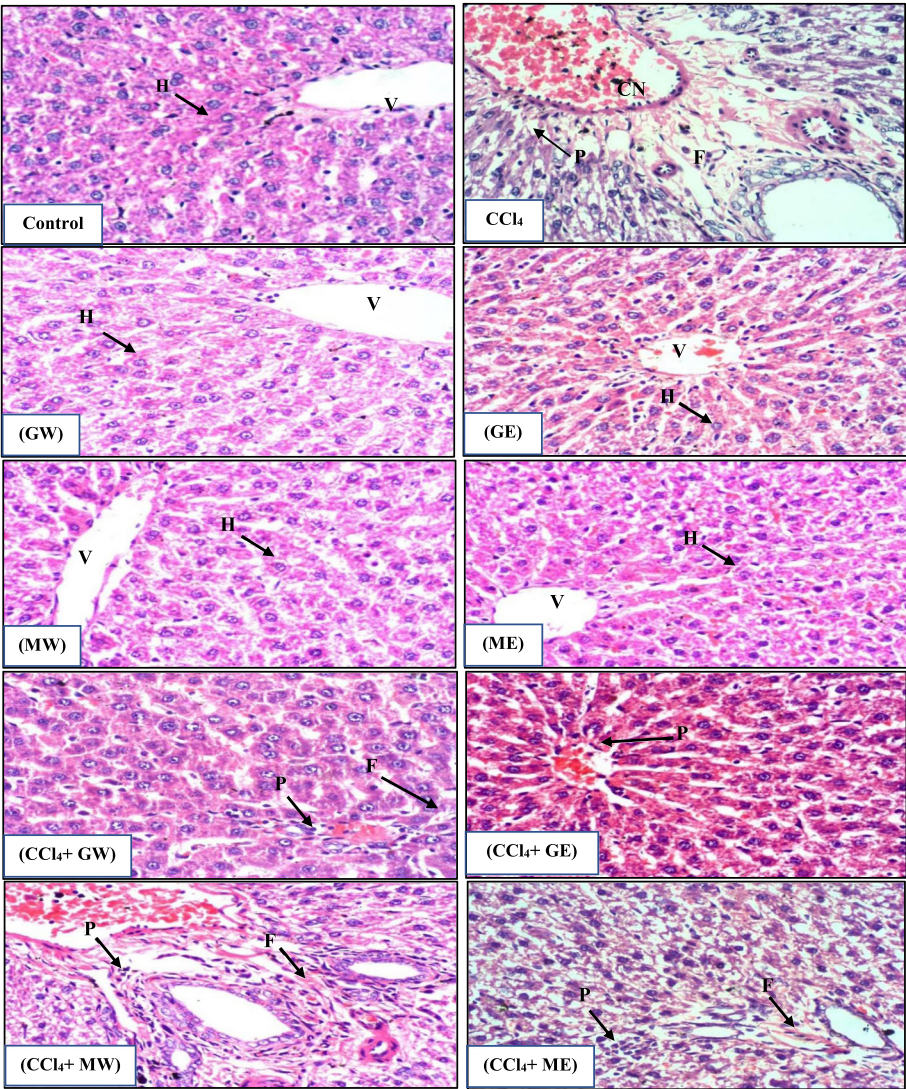
Fig. 4 Photomicrograph of liver stained with H&E showing the following: Normal architecture with the central vein (V) surrounded by hepatocytes (H) (*control group*). Destruction of hepatic architecture with apparent portal fibrosis (F), periportal aggregations of inflammatory cells (P), and marked congestion (Cn) of blood vessels (*CCl₄ group*). Normal architecture of hepatocyte (*GW, GE, MW, and ME groups*), respectively. Few lymphocyte infiltrations and little fibrosis (*CCl₄+ GW group*). Few lymphocyte infiltrations and no fibrosis (*CCl₄+ GE group*). Some fibrosis (F) and lymphatic aggregation(P) (*CCl₄+ MW group*). Few lymphocyte infiltrations (P) and some fibrosis (F) (*CCl₄+ ME group*) (H&E ×400)

Table 5 Summary of the histopathological findings

Groups	Fibrosis	Inflammatory cells infiltration	Congestion of blood vessels
Control	Absent	Absent	Absent
CCl₄	Apparent portal fibrosis	Periportal aggregations of inflammatory cells	Present
GW	Absent	Absent	Absent
GE	Absent	Absent	Absent
MW	Absent	Absent	Absent
ME	Absent	Absent	Absent
CCl₄+GW	Little periportal fibrosis	Few lymphocyte infiltrations	Absent
CCl₄+GE	Absent	Few lymphocyte infiltrations	Absent
CCl₄+MW	Little periportal fibrosis	Few lymphocyte infiltrations	Absent
CCl₄+ME	Little periportal fibrosis	Few lymphocyte infiltrations	Absent

antioxidants GSH and GP_X in the liver tissue which was in accordance with EL-Dakhly et al. (2020). On the other hand, our results prove the protective effects of the tested extracts against CCl_4-induced oxidative stress which is illustrated by amelioration of the depleted levels of GSH and GPx and decreased the levels of hepatic MDA and NO in the CCl_4-treated rats (Table 3).

In the current study, CCl_4-treated rats showed inflammation and hence an overexpression of inflammatory biomarkers (IFN γ, TNF-α, and NF-Kb) in plasma. It was suggested that IFN-γ increases liver injury by stimulating hepatic inflammation and aggravating liver damage; this is accompanied by an increase in hepatic fibrogenesis (Knight, Lim, Yeoh, & Olynyk, 2007). The TNF-α activity also is increased in many forms of experimental liver injury (Riddle et al., 2014). Nuclear Factor κB is a transcription factor that regulates the expression of genes involved in the inflammation process (Sun et al., 2007). These pro-inflammatory cytokines are also involved in the activation of HSCs which have been identified as major collagen-producing cells in the injured liver, playing a role in the production of fibrous tissue and extracellular matrix components (Peng et al., 2009). Hamza et al. (2020) found that repeated administration of CCl_4 caused significant fibrosis of liver cells and release of liver injury markers with a range of inflammatory reactions and oxidative stress.

Our results also showed that the level of plasma adiponectin (adipocytokine) in the CCl_4 intoxicated group was lower than normal; this agreed with the previous studies of Abdel-Moneim, Al-Kahtani, ElKersh, and Al-Omair (2015) and Li et al. (2009). It was indicated that adiponectin level is downregulated in several liver pathological processes, as steatosis, inflammation, and fibrosis (Buechler, Wanninger, & Neumeier, 2011). Adiponectin has many anti-inflammatory activities as it suppresses TNF-α. The anti-inflammatory effects of adiponectin are also exerted by induction of the anti-inflammatory cytokines interleukin-10 (IL-10) or IL-1 receptor antagonist and upregulation of heme-oxygenase-1 (Moschen, Wieser, & Tilg, 2012). Treatment with the natural tested extracts reversed the decrease of adiponectin level induced by CCl_4; moreover, lower levels of IFN-γ, TNF-α, and NF-KB are indicative of improved anti-inflammatory effects and reduction of hepatic inflammation and fibrogenesis (Table 4). We hypothesize that gooseberry and black mulberry extracts might protect the liver from CCl_4-caused injury and fibrogenesis by attenuating oxidative stress and suppressing inflammation.

The histological observations of liver tissues support the results obtained from the biochemical assays. Administration of CCl_4 caused degenerative changes in hepatocytes, including the destruction of hepatic architecture, marked portal fibrosis, activation of hepatic stellate cells, periportal aggregations of inflammatory cells, and congestion of blood vessels; this goes in accordance with the previous studies of Wahid et al. (2016) and Sansoè et al. (2016). On the other hand, administration of our tested extracts along with CCl_4 was able to show an improvement in the liver tissue comparing to the CCl_4 group (Fig. 4).

The hepatoprotective effects of gooseberry and black mulberry extracts are also supported by the previous studies of Arun and Asha (2007), Al-Olayan et al. (2014), and Deniz, Laloglu, Koc, Nadaroglu, and Geyikoglu (2018). Results from Abd El-Rahman, Abd-Elhak, and Zaki (2020) study indicated the efficacy of cape gooseberry and black mulberry as a promising anti-hepatotoxic agent because they have high antioxidant activity.

Conclusion

Our study concluded that the aqueous and ethanolic extracts of gooseberry and black mulberry significantly protect the liver from injury induced by CCl_4 in rats by reducing the biomarkers of liver lesions (ALT, AST, ALP, AFP, and hydroxyproline). Besides, the tested extracts attenuate oxidative stress by increasing the content of hepatic GSH and GPx, leading to a reduction in the levels of MDA and NO. The tested extracts also suppress inflammation by reducing the levels of plasma pro-inflammatory cytokines INF-γ, TNF-α, and NF-KB and elevating the level of plasma adiponectin. Furthermore, the microscopic examination showed improving histological architecture of the liver and reduction of fibrosis. The hepatoprotective effect of the gooseberry and black-mulberry extracts is due to their high content of flavonoids, polyphenols, and their antioxidant activity. Ethanolic extract of gooseberry (GE) showed the most significant hepatoprotective activity followed by ethanolic extract of mulberry (ME).

contributed to the design and conception of the study and preparation of materials required for the study. The two authors read and approved the manuscript.

Availability of data and materials
The datasets analyzed during the present study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The protocol of this study was approved by the research ethical approval committee of the Medical Research Center, Ain Shams University, Cairo, Egypt. The Guidelines and Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. The living conditions of animals were appropriate for their species and contribute to their health and comfort (the housing, feeding, and care of all). The authors ensured that all individuals who deal with animals were under their supervision and received explicit instruction in experimental methods and in the care, maintenance, and handling of the rats. The authors were refining the experimental procedures themselves. They were carefully assessing the method of administration, the effects of the substance on the animal, and the amount of handling and restraint required. All rats were sacrificed using euthanasia to prevent the animals from feeling pain.

Consent for publication
Not applicable

Competing interests
Authors have declared that no competing interests exist.

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