

Hepatoprotective activity of *Brassica oleracea* L. var. *Italica*

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Purpose

This study investigated total ethanol and successive extracts of *Brassica oleracea* L. var. *Italica* inflorescences for their prophylactic and therapeutic effects on rat's liver using the paracetamol-induced hepatotoxicity method. The present investigation focuses on liver histopathological analysis and evaluation of biochemical parameters.

Materials and methods

Hepatoprotective activity of *B. oleracea* L. var. *Italica* was evaluated using the paracetamol-induced hepatotoxicity method on rat's liver. Phytochemical investigation of 80% ethanolic extract was performed using column chromatography and preparative thin layer chromatography. Spectroscopic analyses UV, ¹H-NMR, and ¹³C-NMR together with Electron impact mass spectrum (EIMS) were carried out to identify isolated compounds.

Results

Treatment with total ethanol and different successive extracts of *B. oleracea* showed a significant decrease in the liver enzymes (aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase) when paracetamol was administered before the extracts (therapeutic) or administered after the extracts (prophylactic). On examination of the histopathological results, it is obvious that the prophylactic activity of the extracts was more effective. From ethanol extract, obtucarbamate, *N*-(4-hydroxy phenyl) acetamide, and *p*-hydroxy benzoic acid were isolated using chromatographic methods. The isolated compounds were identified through spectroscopic analysis and compared with literature reports.

Conclusion

Broccoli extracts were studied intensively for their prophylactic and therapeutic effects on rat's liver damage induced by paracetamol. The evaluation of biochemical parameters and histopathological analysis showed that broccoli extracts have prophylactic activity against liver damage. Phytochemical investigation of 80% ethanolic extract resulted in isolation of three compounds, obtucarbamate, *N*-(4-hydroxy phenyl) acetamide, and *p*-hydroxy benzoic acid, and can be considered a good candidate for the protection of liver damage induced by paracetamol.

Keywords:

Brassica oleracea, hepatoprotective, obtucarbamate

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Introduction

Diet is the single greatest contributor toward human cancer and may be associated with 35–70% of the incidence of the disease. Although various carcinogens are present in foods, their effects are minor compared with dietary components that inhibit the cancer process.

A plant-based diet is widely suggested to contribute toward reducing the risk of development of chronic diseases such as cancer, atherosclerosis, cardiac dysfunctions, diabetes, hypertension, and neurodegenerative disorders [1–5]. This function is largely because of the antioxidant effect of their bioactive components. One of the common denominators in the pathogenesis of most chronic diseases is the implication of oxidative stress mechanisms [6–8].

Uridine and uridine 9-acetate were isolated from the ethyl acetate extract of *Brassica oleracea* var. *Italica*

(Brassicaceae). Ethyl acetate extracts have been found to be effective against *Bacillus cereus*, *Escherichia coli*, and *Candida albicans* [9].

By liquid chromatography–electrospray ionization mass spectrometry, two glucosinolates, glucoiberin and 3-hydroxy, 4(α-L-rhamnopyranosyloxy) benzyl glucosinolate, were identified in the aqueous extract of *B. oleraceae* L. var. *Italica*. Further, two compounds were isolated after enzymatic hydrolysis of the aqueous extract by myrosinase. When myrosinase hydrolysate was tested for cytotoxic activity on the colon cancer cell line, it showed very high activity – 95% lethality up to 0.78 μg/ml [10].

GC/MS analysis of the volatiles produced by the action of the endogenous cystine lyase in *B. oleraceae* L. var. *Italica* florets showed three sulfur compounds: disulfide dimethyl, trisulfide dimethyl, and 1,2,4-trithiolane 3,5 dimethyl, whereas because of the enzymatic hydrolysis

of endogenous myrosinase of broccoli florets, four isothiocyanate compounds were produced. Chloroform extract, combined ethyl acetate and ethanol, and crude extract of broccoli was shown to cause significant loss in the body weight of female rats. Testing of the crude extract and successive extracts isolated from *B. oleracea* for antioxidant activity by inhibiting the stable DPPH free radical, chloroform, and successive ethanol extracts showed 100% activity compared with ascorbic acid [11].

The present study was carried out to determine the bioactivity of different extracts of broccoli on liver cells as a prophylactic and therapeutic agent.

Materials and methods

Apparatus

¹H-NMR spectrophotometer, Jeol EX 500 NMR spectrometer, Tokyo -Japan.

¹³C-NMR spectrophotometer, Jeol EX 125 spectrometer.

A mass spectrophotometer, a Hewlett-Packard 5870, United State was used at an oven temperature 60–200°C at a rate of 3°C/min; the injection port temperature was 150°C and the split ratio was 100 : 1. A mass selective detector was also used; the carrier flow was 1 ml/min; ionization voltage was 70 eV; and start–stop masses were 35–500.

Systems

S1	<i>n</i> -Butanol : acetic acid : water	3 : 1 : 1
S2	Acetic acid : water	15 : 85
S3	Chloroform : methanol	9 : 1
S4	Benzene : ethyl acetate	9 : 1
S5	Chloroform	100%

Plant material

Broccoli florets were used, produced from the National Research Centre farm in Noharia, in March 2009.

Preparation of crude extract

Fresh broccoli florets (1 kg) were extracted exhaustively with 80% ethanol (total ethanol extract) in a Soxhlet apparatus, Germany. The extract produced was dried at 40°C under vacuum.

Preparation of successive extracts

Fresh broccoli florets (1 kg) were successively extracted in a Soxhlet apparatus using petroleum ether, chloroform, ethyl acetate, and then 95% ethanol.

Isolation of compounds I, II, and III

Compound I was isolated from ethanol extract using the pc technique, Whatmann No. 3 MM sheets, Kent, UK and the solvent system S1. Further purification was carried out using a silica gel column and eluted with chloroform, and polarity increased gradually with methanol. Compound I was collected as a white amorphous powder and was then subjected to spectroscopic analysis.

The crude extract (80% ethanol extract) was concentrated and treated with an equal volume of a 10% KOH solution at room temperature for 1 h. The alkaline alcoholic extract was diluted with water and extracted with ether. The aqueous layer was acidified with dilute HCl, refluxed for 1.5 h, cooled, and extracted with ether, whereby the ethereal extract was evaporated to dryness.

The previous dried extract was placed in a silica gel, Merck, Germany column and developed with S4: two fractions were collected: F1 and F2. F1 was subjected to preparative thin layer chromatography using silica gel G60, F254-precoated plates, developed with solvent system S3 to yield compound II. F2 was placed in a silica gel column and eluted with solvent system S5, which yielded compound III.

Characterization of compounds

Hepatoprotective activity of Brassica oleracea L. var. Italica

The study aimed to detect the protection and curative effects of petroleum ether, chloroform, ethyl acetate combined with ethanol extract, and total ethanol extracts of *B. oleracea* var. *Italica* against the hepatotoxicity induced by paracetamol.

Experimental design

A total number of 78 female rats (120–150 g) were used in this study. Rats were obtained from the Laboratory Animal House, National Research Centre, Cairo, Egypt. They were acclimatized to the new laboratory conditions before use in experimentation for a period of 1 week in a quiet room at a temperature of 20–25°C with a 12 h light and 12 h dark cycle. Rats were provided with food and water *ad libitum* throughout the period of the experiment. All animal procedures in this study were approved by the Ethics Committee for the use of nonhuman animals in biological research of the National Research Centre, Cairo, Egypt.

Doses

Doses of the drugs were based on the human dose after conversion into that of rats and mice according

to the method by [12] and were administered orally by a gastric tube. Doses of the tested extracts were adjusted to be (1/10 of LD50). The LD50 test on the crude extract of broccoli florets showed that oral administration of up to 10 g/kg body weight is safe and did not kill any of the tested animals; thus, it could be administered at high doses without any toxicity [11].

Rats were divided into 13 groups (six rats/group):

Group I

The animals of this group served as a control group and were treated with 1 ml of vehicle for 8 days.

Group II (negative control)

Rats received an oral dose of vehicle for 7 days before a single oral dose of paracetamol (2 g/kg body weight) on the eighth day [13].

Group III (positive control)

Rats received an oral dose of silymarin (50 mg/kg body weight) for 7 days before a single dose of paracetamol (2 g/kg body weight) [13].

Groups IV, V, VI, VII

Animals received petroleum ether, chloroform, ethyl acetate combined with ethanol and total ethanol extracts of *B. oleracea* var. *Italica* (1 g/kg body weight) for 7 days before a single dose of paracetamol (2 g/kg body weight) on the eighth day.

Group VIII

Rats received an oral dose of vehicle for 7 days after a single dose of paracetamol (2 g/kg body weight) [13].

Group IX

Rats received an oral dose of silymarin (50 mg/kg body weight) for 7 days after a single dose of paracetamol (2 g/kg body weight).

Groups X, XI, XII, XIII

Animals received petroleum ether, chloroform, ethyl acetate combined with ethanol, and total ethanol extracts of *B. oleracea* var. *Italica* (1 g/kg body weight) for 7 days after a single dose of paracetamol (2 g/kg body weight).

Paracetamol and silymarin solutions were prepared by dissolving in distilled water. All extracts were dissolved in water as a vehicle, except petroleum ether extract, which was dissolved in oil.

Biochemical study

At the end of the experiment, blood samples were obtained using the orbital sinus technique [14]. Blood was collected in a dry tube and left to clot at room temperature, and then centrifuged at 3000 rpm for 10 min. Serum was separated and kept for subsequent evaluation of biochemical parameters.

Determination of serum glutamate pyruvate transaminase (SGPT), glutamate oxaloacetate transaminase (SGOT), and alkaline phosphatase (ALP) was carried out using colorimetric methods [15].

Histopathological study

After blood samples were obtained, rats were decapitated and slices of the liver were removed and fixed instantaneously in formal saline for 24 h. The specimens were washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Sections of 6 mm thickness were prepared and stained with hematoxylin and eosin [16]. The prepared sections of the liver were examined by light microscopy.

Statistical analysis

All values obtained were evaluated as mean \pm SE and the statistical significance of differences between the means was determined using the SPSS for Windows, version 11.0 statistical program (SPSS Inc., Chicago, Illinois, USA). For all statistical evaluations, *P* values less than 0.05 were considered as statistically significant.

Results and discussion

Identification of compounds

Compound I, EIMS, showed the molecular ion peak at 238 for the CR11RH14RNR2O4 ¹H-NMR spectrum. Table 1 shows a doublet signal at δ 7.1 ppm, attributed to 1H-6, *J*=8.4 Hz, which was also achieved by ²*PJ* coupling with C-1, 4 and ³*PJ* coupling with C-2, 4, and 7. 1H-5 appeared as a dd signal at δ 7.19 ppm, *J*=1.15, 8.4 Hz; this proton is also recognized by ²*PJ* coupling with C-4 and ³*J* coupling with C-1, 3. 1H-3 appeared as a broad singlet at δ 7.5 ppm, which is further achieved by ²*PJ* coupling with C-2, 4 and ³*PJ* coupling with C-1 and C-5. 3H-7 appeared as a singlet at δ 2.22 ppm, and is recognized by ²*PJ* coupling with C-1 and ³*J* coupling with C-2 and C-6.

3H-9 and 3H-11 appeared as a singlet at δ 3.73 ppm and 3H-9 was achieved by ²*J* coupling with C-8 and 3H-11 with C-10.

The N–H at position 12 appeared as a singlet at δ 8.8 ppm and was achieved by 2J coupling with C-4 and 3J coupling with C-5 and 3. The other N–H at position 13 also appeared as a singlet at δ 8.6 ppm and was further achieved by 2PJ coupling with C-1, 8 and 3J coupling with C-3.

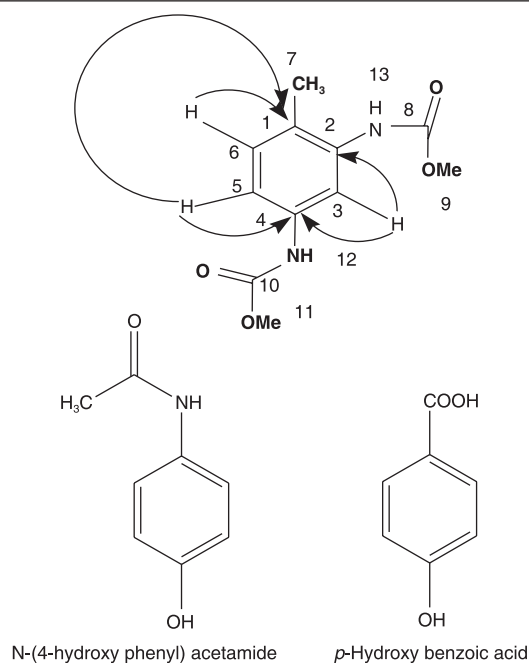
The quaternary carbons were correlated through the heteronuclear multi-bond coherence (HMBC) spectrum (2J – 3PJ) (Fig. 1).

The attached proton set (APT) spectrum showed five even and five odd carbon atoms, with the odd carbon at δ 51.2 ppm corresponding to C-9 and C-11. C-7 also appeared downward at δ 17.2 ppm; C-6, 5, and 3 appeared downward at δ 131.6, 117.5, and 116.9 ppm, respectively. However, C-2, 1, and 4 appeared upward at δ 137.5, 125.1, and 138.7 ppm, respectively. C-8 and 10 also appeared upward at δ 152.1 and 157.4 ppm, respectively.

The heteronuclear single quantum coherence (HSQC) spectrum proved the integrity of the assumed structure as it determined the connectivity of H–C for the entire compound. Thus, compound I was identified as obtucarbamate (*N,N'*-bis(methoxycarbonyl)-2,4-diaminotoluene) isolated previously from the bark of *Chamaecyparis obtusa* Fam. Cupressaceae [17].

Compound II was identified as *N*-(4-hydroxy phenyl) acetamide from $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ together with EIMS.

Figure 1



HMBC of obtucarbamate. *N*-(4-hydroxy phenyl) acetamide *p*-Hydroxy benzoic acid.

N-(4-hydroxy phenyl) acetamide fx1

Compound III was identified as *p*-hydroxy benzoic acid from $^1\text{H-NMR}$ together with EIMS.

p-Hydroxy benzoic acid fx2

Biochemical results

Prophylactic test

SGPT: A significant increase ($P < 0.05$) in the SGPT level was found in the paracetamol-treated group compared with the control group. A significant decrease ($P < 0.05$) was found in the SGPT level of the silymarin+paracetamol, petroleum ether extract+paracetamol, chloroform extract+paracetamol, ethylacetate combined with ethanol extract+paracetamol, and total ethanol extract+paracetamol compared with the paracetamol group (Table 2).

SGOT: The results showed a significant increase ($P < 0.05$) in the SGOT level in the paracetamol

Table 1 $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, HSQC, and HMBC data of compound I

	HSQC		HMBC	
	$^1\text{H-NMR}$ (500 MHz, DMSO)	APT (125 MHz, DMSO)	2J	3J
–	–	125.1	–	–
–	–	137.5	–	–
7.5, Broad singlet	–	116.9	137.5, 138.7	125.1, 117.5
–	–	138.7	–	–
7.19 (dd, $J = 1.15, 8.4$ Hz)	–	117.5	138.7	116.9, 125.1
7.1 (d, $J = 8.4$ Hz)	–	131.6	117.5, 125.1	138.7, 137.5, 17.2
2.22, Singlet	–	17.2	125.1	137.5, 131.6
–	–	152.1	–	–
3.7, Singlet	–	51.2	152.1	–
–	–	157.4	–	–
3.7, Singlet	–	51.2	157.4	–
8.8, Singlet	–	–	138.7, 157.4	117.5, 116.9
8.6, Singlet	–	–	137.5, 152.1	116.9

DMSO, dimethyl sulfoxide.

Table 2 Liver function of rats administered different extracts of broccoli and paracetamol (prophylactic test)

Groups	Parameters		
	GPT (IU/l)	GOT (IU/l)	ALP (IU/l)
Group I	30.2 \pm 3.8	93 \pm 1.6	65.5 \pm 1.2
Group II	112.8 \pm 1.8*	183.1 \pm 2.8*	195.6 \pm 4.5*
Group III	60.6 \pm 1.1**	94.2 \pm 2.6**	98.1 \pm 20.8**
Group IV	70.7 \pm 1.2**	116.1 \pm 6.5**	101.8 \pm 6.1**
Group V	79.0 \pm 4.1**	124.7 \pm 2.9**	124.1 \pm 8.3**
Group VI	69.9 \pm 4.7**	122.1 \pm 4.3**	116.6 \pm 6.1**
Group VII	74.5 \pm 4.2**	133.7 \pm 8.4**	119.9 \pm 3.4**

Data presented as means \pm SE; ALP, alkaline phosphatase; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; *Significant at $P < 0.05$ as compared with the control group; **Significant at $P < 0.05$ as compared with the paracetamol group.

group compared with the control group, whereas the silymarin+paracetamol, petroleum ether extract+paracetamol, chloroform extract+paracetamol, ethylacetatecombinedwithethanol extract+paracetamol, and total ethanol extract+paracetamol groups showed a significant decrease in the SGOT level compared with the paracetamol group (Table 2).

ALP: In case of the paracetamol group, a significant increase ($P < 0.05$) in the serum ALP was found compared with the control group. However, the silymarin+paracetamol, petroleum ether extract+paracetamol, chloroform extract+paracetamol, ethyl acetate combined with ethanol extract+paracetamol, and total ethanol extract+paracetamol showed a significant decrease in the level of serum ALP compared with the paracetamol group (Table 2).

Therapeutic test

SGPT: The paracetamol group showed a significant increase in the level of SGPT compared with the control group. However, the following groups: paracetamol+silymarin, paracetamol+petroleum ether extract, paracetamol+chloroform extract, paracetamol+ethyl acetate combined with ethanol extract, and paracetamol+total ethanol extract groups showed a significant decrease in the level of SGPT compared with the paracetamol groups (Table 3).

SGOT: Serum GOT obtained from the paracetamol group showed a significant increase compared with the control one. The comparison of the above results with the paracetamol group revealed a significant decrease ($P < 0.05$) in the SGOT level for the paracetamol+silymarin, paracetamol+petroleum ether extract, paracetamol+chloroform extract, paracetamol+ethyl acetate combined with ethanol extract, and paracetamol+total ethanol extract groups (Table 3).

ALP: In case of the paracetamol group, a significant increase ($P < 0.05$) in the serum ALP was found compared with the control group. However, the paracetamol+silymarin, paracetamol+petroleum ether extract, paracetamol+chloroform extract, paracetamol+ethyl acetate combined with ethanol extract, and paracetamol+total ethanol extracts showed a significant decrease in the serum ALP level compared with the paracetamol group (Table 3).

Histopathological results

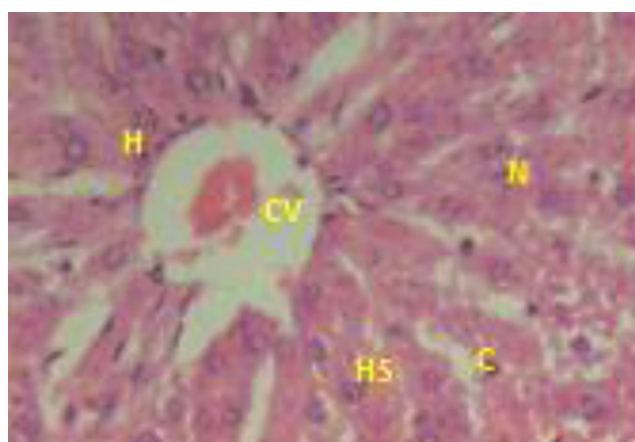
The hepatic lobules are the structural units of the liver; each is composed of cords of hepatocytes and blood sinusoids in between (Fig. 2).

Table 3 Liver functions of rats administered a single dose of paracetamol and treated with different extracts of broccoli (therapeutic test)

Groups	GPT (IU/l)	GOT (IU/l)	ALP (IU/l)
Control	30.2 ± 3.8	93.7 ± 10.6	60.5 ± 2.2
Group VIII	129.0 ± 6.8*	213.4 ± 14.4*	135.6 ± 10.5*
Group IX	71.4 ± 2.5**	126.8 ± 8.5**	89.4 ± 3.7**
Group X	88.9 ± 5.3**	131.9 ± 5.6**	93.3 ± 4.8**
Group XI	77.7 ± 5.2**	129.6 ± 4.9**	97.5 ± 2.1**
Group XII	80.0 ± 5.8**	134.2 ± 4.6**	100.6 ± 7.4**
Group XIII	87.8 ± 5.3**	141.6 ± 6.3**	96.9 ± 6.4**

Data presented as means ± SE; ALP, alkaline phosphatase; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; *Significant at $P < 0.05$ as compared with the control group; **Significant at $P < 0.05$ as compared with the paracetamol group.

Figure 2



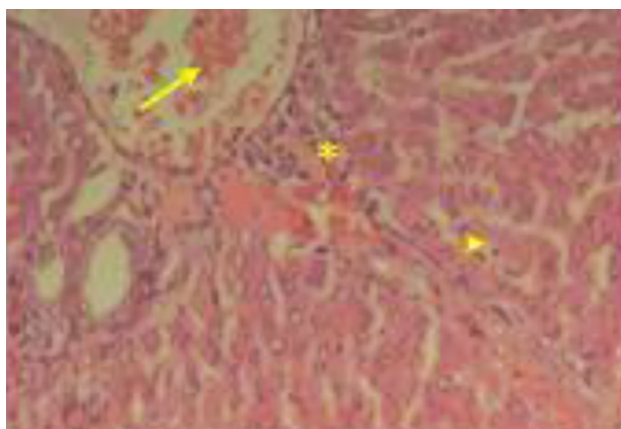
Histopathological examination of liver hepatic lobules.

Histopathological examination of the liver of rats after 24 h of administration of one dose of paracetamol equivalent to 2 g/kg showed mild lymphocytic infiltration in the portal and periportal areas, which was associated with dilatation and congestion of the veins (long arrow). Notice, some nuclei are pyknotic (Fig. 3). However, widespread swelling and ballooning hepatocytes, focal necrosis, and slight hemorrhage were observed (Fig. 4).

Oral administration of silymarin at a dose of 50 mg/kg orally for 7 days, followed by one dose of paracetamol equivalent to 2 g/kg on the eighth day showed that the hepatocytes of the treated rats appeared more or less normal. Dilatation of the hepatic sinusoids was found (Fig. 5). In some rats, slight inflammatory infiltration was present in the portal and periportal tract (Fig. 6).

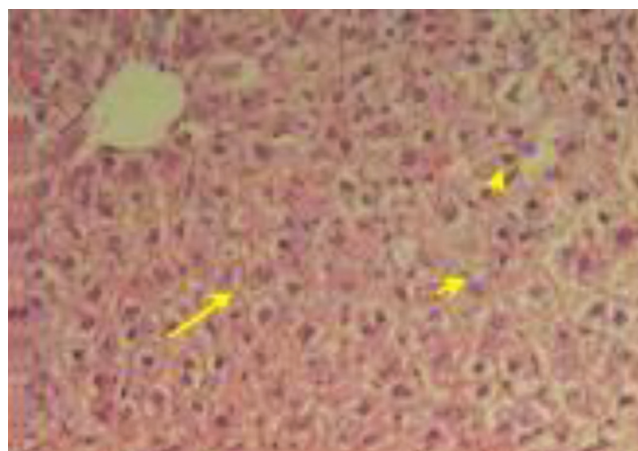
In rats that received daily doses of petroleum ether extract of *B. oleracea* at a dose of 1 g/kg body weight orally for 7 days and administered one dose of

Figure 3



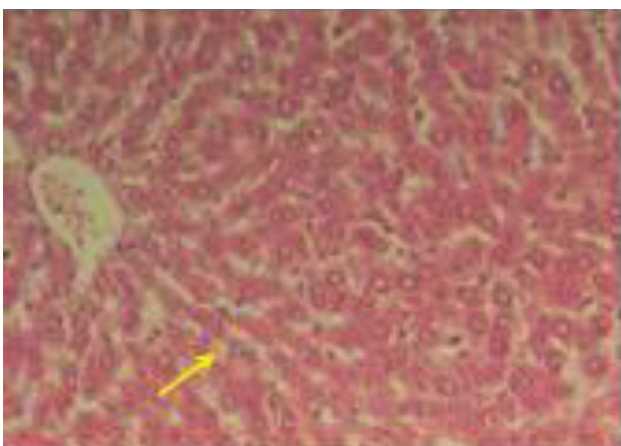
Histopathological examination of liver nuclei.

Figure 4



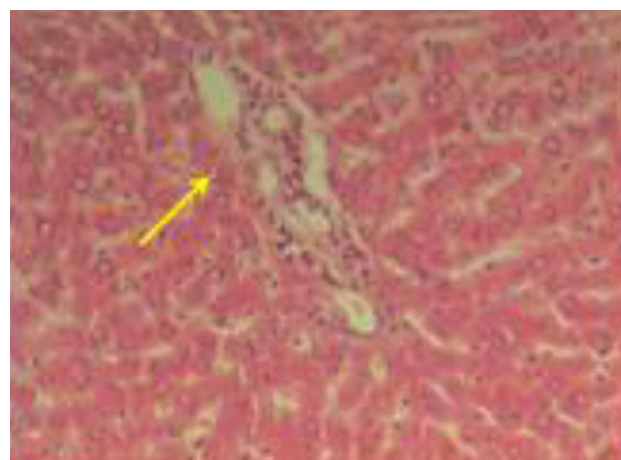
Histopathological examination of liver hepatocytes.

Figure 5



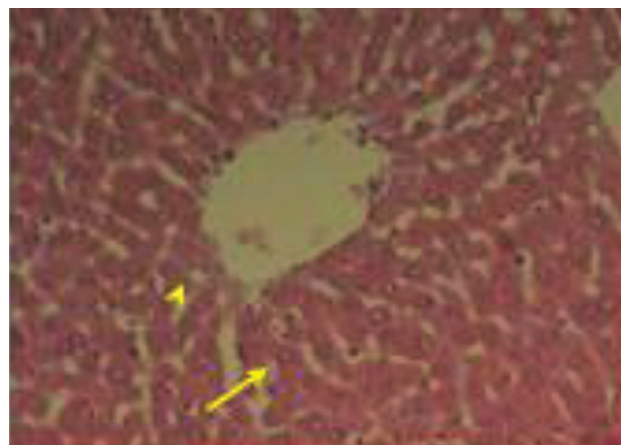
Histopathological examination of liver dilatation of the hepatic sinusoids (administration of Silymarin).

Figure 6



Histopathological examination of liver of portal and periportal tract.

Figure 7



Histopathological examination of liver hepatic sinusoids of petroleum ether extract.

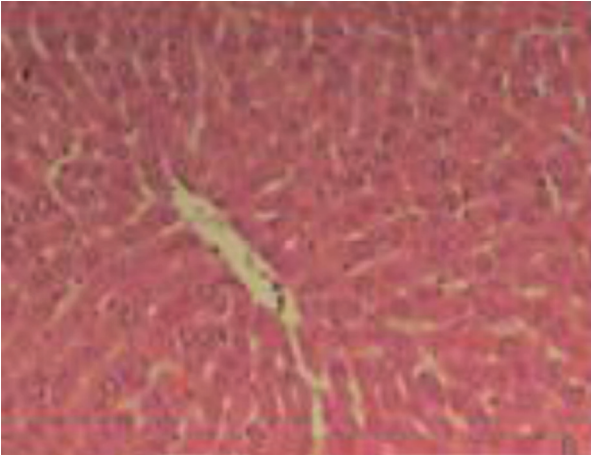
paracetamol equivalent to 2 g/kg on the eighth day, the liver showed a normal structure. Dilatation of

the hepatic sinusoids and a few vacuoles was present (Fig. 7). In some rats, the architecture of the hepatic lobules appeared more or less like the control one (Fig. 8).

Examination of the liver of rats administered an oral dose (1 g/kg body weight) of CHCl₃ extract of *B. oleracea* for 7 days and administered one dose of paracetamol (2 g/kg) on the eighth day showed a normal structure. Activated Koppfer cells were observed (Fig. 9). However, the liver of some rats showed focal necrosis and some vacuoles (Fig. 10).

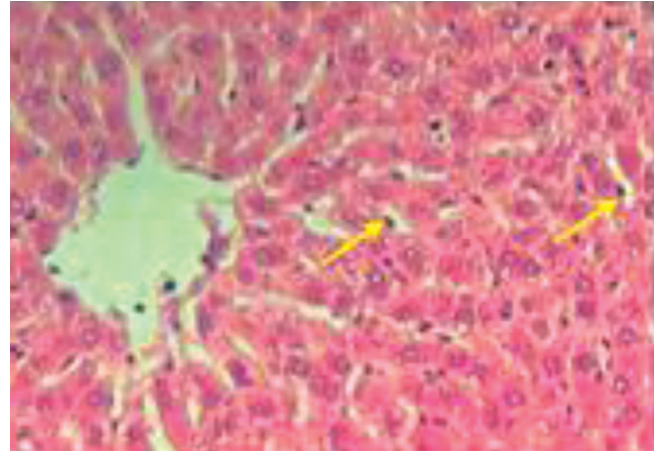
Microscopic investigation of the liver of rats administered an oral dose of ethyl acetate extract of *B. oleracea* (1 g/kg body weight) for 7 days, followed by one dose of paracetamol equivalent to 2 g/kg on the eighth day showed a normal structure, except the slight inflammatory infiltration beside the central veins (Fig. 11).

Figure 8



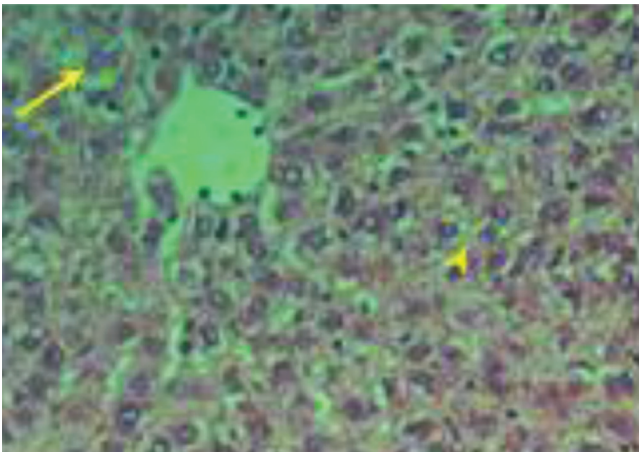
Histopathological examination of liver architecture of hepatic lobules (petroleum ether extract).

Figure 9



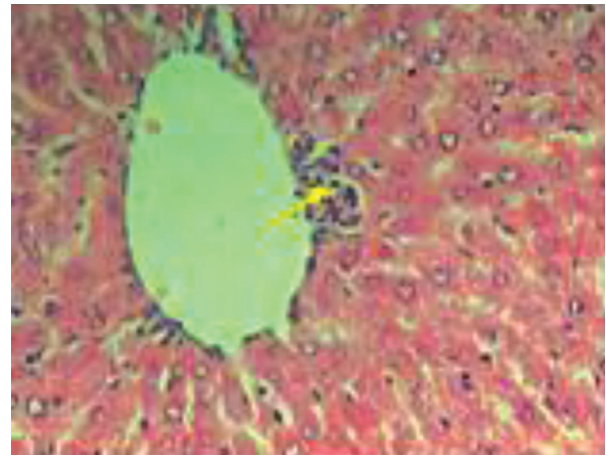
Histopathological examination of liver activated Koppfer cells (CHCl_3 extract).

Figure 10



Histopathological examination of liver chloroform extract.

Figure 11



Microscopic investigation of liver rats cells for ethyl acetate extract.

The liver of rats administered daily doses of total ethanol extract of *B. oleracea* at a dose of 1 g/kg body weight orally for 7 days and administered one dose of paracetamol equivalent to 2 g/kg on the eighth day showed dilatation of hepatic sinusoids and slight inflammatory infiltration (Fig. 12).

Histopathological examinations of the liver of rats administered one dose of paracetamol equivalent to 2 g/kg, followed by an oral dose of petroleum ether extract of *B. oleracea* at a dose of 1 g/kg body weight for 7 days showed swelling degeneration in the hepatocytes and congestion in the central vein in some rats (Fig. 13). In addition, the liver of other rats showed a normal structure. Dilatation of some hepatic sinusoids was observed (Fig. 14).

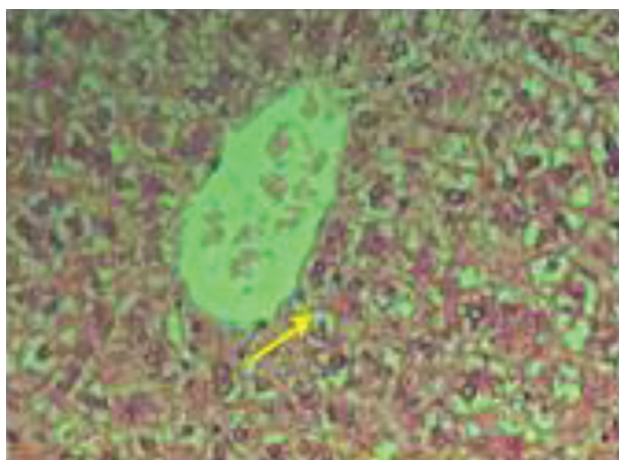
Examination of sections of the liver of rats administered one dose of paracetamol equivalent to

2.5 g/kg, followed by a daily dose of received CHCl_3 extract of *B. oleracea* at a dose of 1 g/kg body weight orally showed vacuoles in the hepatocytes (Fig. 15).

The liver of rats administered one dose of paracetamol equivalent to 2 g/kg, followed by a daily dose of ethyl acetate extract of *B. oleracea* at a dose of 1 g/kg body weight orally showed few vacuoles in the hepatocytes (Fig. 16).

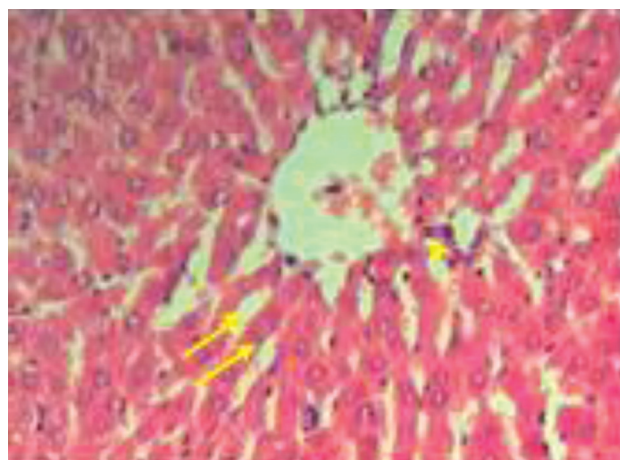
Examination of liver sections of rats that received a single oral dose (Fig. 17). A micrograph of the liver of rats administered one dose of paracetamol equivalent to 2.5 g/kg, followed by a daily oral dose of total ethanol extract of *B. oleracea* at a dose of 1 g/kg body weight showing swelling degeneration in many hepatocytes. Few of the hepatocytes appeared more or less normal.

Figure 12



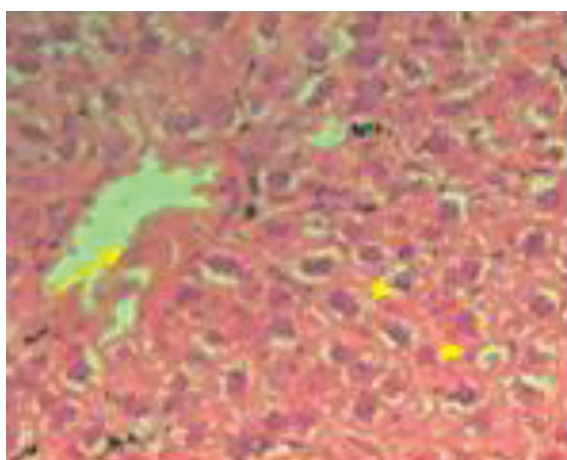
Microscopic investigation of liver rat cells of ethanol extract.

Figure 13



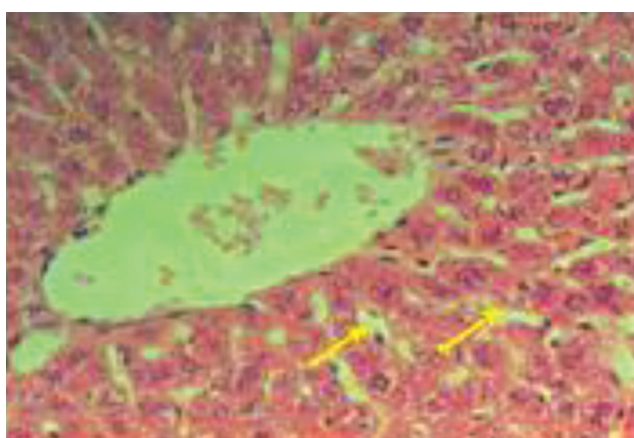
Microscopic investigation of liver rats cells for petroleum ether extract.

Figure 14



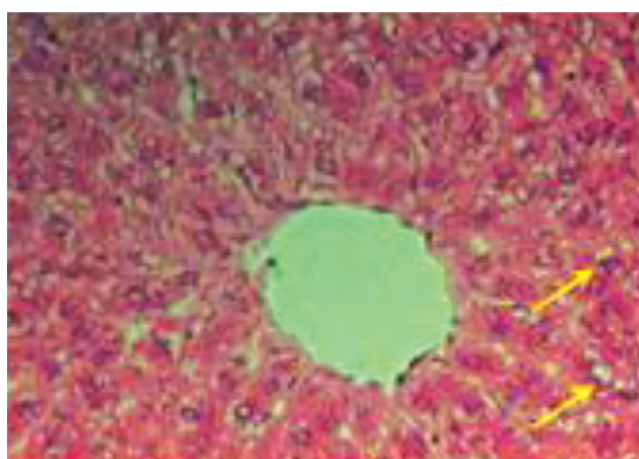
Microscopic investigation of other liver rat cells showed normal structure for petroleum ether extract.

Figure 15



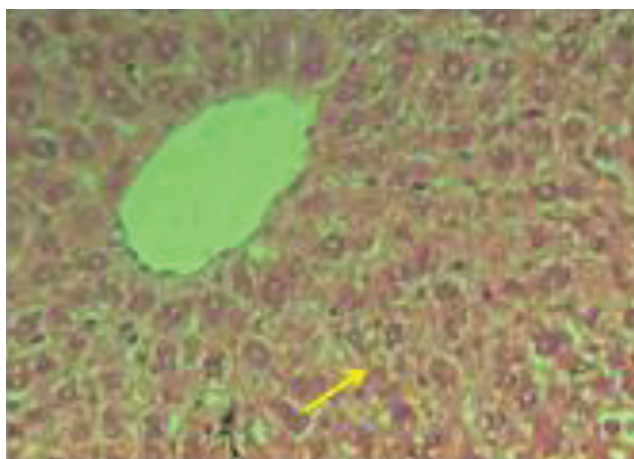
Microscopic investigation of liver of rats cells for chloroform extract.

Figure 16



Microscopic investigation of liver rat cells showed few vacuoles in the hepatocytes of ethyl acetate extract.

Figure 17



Microscopic investigation of liver sections of rats received a single oral dose of paracetamol followed by a daily oral dose of total ethanol extract.

Conclusion

The results showed that administration of paracetamol causes severe acute liver damage in rats, indicated by a marked increase in serum GPT and GOT levels. The increased serum levels of GPT and GOT have been attributed to the damaged structural integrity of the liver. It was also found from the biochemical parameters that all the extracts showed a significant decrease in the liver enzymes when paracetamol was administered before the extracts (treatment) or after the extracts (prophylactic).

On examining the histopathological results, it could be concluded that the prophylactic activity of the extracts was better than the curative effect. It was obvious in case of pretreatment of rats with different extracts (1 g/kg) for 7 days before a single dose of paracetamol (2 g/kg) that most of the hepatic lobules appeared more or less normal, especially petroleum ether extract, ethyl acetate combined with ethanol extract, and chloroform extract, which confirm the results obtained from the biochemical parameters. For the rats that were administered a single dose of paracetamol (2 g/kg), followed by the extracts (1 g/kg) for 7 days, the tested extracts showed swelling degeneration in the hepatocytes and some other rats showed congestion in the central vein. Thus, it can be concluded that broccoli shows good hepatoprotective activity against liver damage.

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Conflicts of interest

There are no conflicts of interest.

References

- 1 Forney CF, Mattheis JP, Austin RK. Volatile compounds produced by broccoli under anaerobic conditions. *J Agric Food Chem* 1991; 39:2257–2259.
- 2 Chin HW, Lindsay RC. Mechanisms of formation of volatile sulphur compounds following the action of cysteine sulfoxide lyases. *J Agric Food Chem* 1994; 42:1529–1536.
- 3 Di Pentima H, Rios JJ, Clemente A, Olias JM. Biogenesis of off-odor in broccoli storage under low-oxygen atmosphere. *J Agric Food Chem* 1995; 43:696–6993.
- 4 Dan K, Todoriki S, Nagata M, Yamashita I. Formation of volatile sulphur compounds in broccoli stored under anaerobic condition. *J Jpn Soc Hort Sci* 1997; 65:867–875.
- 5 Forney CF, Jordan MA. Anaerobic production of methanethiol and other compounds by Brassica vegetables. *Hort Sci* 1999; 34:696–699.
- 6 Hansen M, Butery RG, Stern DG, Cantwell MI, Ling LC. Broccoli storage under low-oxygen atmosphere: identification of higher boiling volatiles. *J Agric Food Chem* 1992; 40:850–852.
- 7 Bailey GS, Williams DE. Potential mechanisms for food related carcinogens and anticarcinogens. *Food Technol* 1993; 47:105–118.
- 8 Fahey JW, Zhang Y, Talalay P. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc Natl Acad Sci USA* 1997; 94:10367–10372.
- 9 Hashem FA, Motawea MH, El-Shabrawy AE, Shaker K, El-Sherbini S. Myrosinase hydrolysates of *Brassica oleraceae* L. Var. *italica* reduce the risk of colon cancer. *Phytother Res* 2012a; 26:743–747.
- 10 Hashem FA, Motawea MH, El-Shabrawy AE, Shaker K, El-Sherbini S. *Brassica oleracea* var. *italica*: a nutritional supplement with antimicrobial potential. *J Herbs Spices Med Plants* 2012b; 18:93–100.
- 11 Motawea MH, Hashem FA, El-Shabrawy AE, El-Sherbini S. *Brassica oleraceae* L var. *italica*: a nutritional supplement for weight loss. *Aust J Med Herbal* 2010; 22:127–131.
- 12 Paget G, Berne's E. Laurence DR, Bacharach AL, ed.. Toxicity tests in evaluation of drug activities 'cited in the laboratory rat'. Eds. Laurence, D.R, and Bacharach, A.L., London: Academic Press; 1964. 135–160.
- 13 Mitra SK, Venkataranganna MV, Sundaram R, Gopumadhavan S. Protective effect of HD-03, a herbal formulation, against various hepatotoxic agents in rats. *J Ethnopharmacol* 1998; 63:181–186.
- 14 Sanford HS. Method for obtaining venous blood from the orbital sinus of the rat or mouse. *Science* 1954; 119:100.
- 15 Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol* 1957; 28:56–63.
- 16 Drury RB, Wallington EA. *Carleton's histopathological technique*. 4th ed.. New York, Oxford University Press; 1980.
- 17 Kuo YH, Jou MH. Two carbamates, obtucarbamate A and B from the bark of *Chamaecyparis obtusa*. *Chem Express* 1990; 5:909–912.