Antiproliferative activity of Brassica nigra seeds extract in liver tissue of mice exposed to phenobarbital

Ibtehal Naseer Salman*, Dalya Basil Hanna, Bahir Abdul-Razzaq Mshimesh ***

Department of Pharmacology/ College of Pharmacy/ Mustansiriyah University, Baghdad, Iraq.

****** Department of Clinical Laboratory Sciences/ College of Pharmacy/ Mustansiriyah University, Baghdad, Iraq***

Article Info:

Received Feb 2022
Accepted Apr 2022
Corresponding Author email:
dr.bahirrazzaq@uomustansiriyah.edu.iq
orcid: https://orcid.org/ 0000-0003-4421-8690

DOI:

Abstract:

Hepatocellular proliferation is one of the most common causes of hepatocellular carcinoma (HCC), a type of cancer that is widely distributed disease. Hepatocellular carcinoma treatment has numerous barriers, including ineffectiveness, side effects, and drug resistance to currently available treatments. Previous studies showed that a high intake of Brassica vegetables has been associated to a decreased risk of a number of malignancies. The aim of this study is the evaluation of antiproliferative activity of Brassica nigra seeds extract in mice exposed to phenobarbital. Brassica nigra seeds where extracted; phytochemical analysis of the extract was done that including phytochemical screening tests and Gas chromatography-mass spectrometry (GC-MS) analysis. Antiproliferative activity of hydro alcoholic Brassica seeds extract has been studied by 800mg/kg and compare with control group (given normal saline), phenobarbital group (Phenobarbital 75mg/kg) and combination group (Brassica extract 800mg/kg+ Phenobarbital 75mg/kg). The GC-MS analysis revealed the presence of isothiocynate compound. Histologically phenobarbital induced severe hepatocellular proliferation (hyperplasia and hypertrophy), glass ground cytoplasm, while Brassica seeds extract produce improvement in histopathological changes that include mild scattered proliferation picture and eosinophilic cytoplasm. In comparison to phenobarbital group, Combination groups pretreated with Brassica nigra seeds for 14 days and phenobarbital for 7 days caused significant reduction relative liver weight and alanine aminotransferase (ALT) Brassica nigra seeds extract have isothiocynate as main compound it showed antiproliferative action on the liver tissue, implying that it may have a promising effect in minimizing the risk of liver cancer.

Key words: Hepatocellular proliferation, Phenobarbital, Brassica seeds.

تقييم فاعلية مستخلصات البراسيكا السوداء على تكاثر خلايا الكبد في نموذج الفئران المختبرية

ابتهال نصير سلمان*، داليا باسل حنا **، باهر عبد الرزاق مشيمش*

**** كلية الصيدلة/الجامعة المستنصرية/فرع الادوية والسموم***

***** كلية الصيدلة/الجامعة المستنصرية/فرع العلوم المختبرية السريرية***

الخلاصة:

يعتبر تكاثر الخلايا الكبدية أحد الأسباب الرئيسية للاصابة بسرطان الكبد (HCC) وهو نوع منتشرعلى نطاق واسع مصحوبا بمعدل وفيات مرتفع نسبيا. هناك تحديات كبيرة في علاج سرطان الخلايا الكبدية، بما في ذلك عدم الفعالية،

الآثار الضارة، والمقاومة الدوائية للعلاجات المتوفرة. من خلال الادبيات السابقة، ثبت أن تناول كميات كبيرة من خضروات العائلة النباتية البراسيكا ارتبط بانخفاض مخاطر الإصابة بالعديد من أنواع السرطان.
الهدف من هذه الدراسة هو تقييم تأثير مستخلص بذور براسيكا نيجرا(الخردل الاسود) ضد تكاثر الخلايا الكبدية الناجم عن الفينوباربيتال كعامل محفز في الفئران .
تم تحضير مستخلص بذور براسيكا نيجرا(الخردل الاسود)، بعد ذلك ، تم إجراء التحليل الكيميائي النباتي وطيف الكتلة اللونية للغازبواسطة جهاز .GC-MS
تمت دراسة النشاط المضاد للتكاثر لمستخلص بذور براسيكا الكحولي المائي بمقدار 800 مجم / كجم ومقارنته بمجموعة التحكم (بمحلول ملحي طبيعي) ومجموعة الفينوباربيتال (فينوباربيتال 75 مجم / كجم) والمجموعة المركبة (مستخلص براسيكا 800 مجم / كجم + فينوباربيتال 75 مجم / كجم). كشف تحليل GC-MS عن وجود مركب ايزوثايوسايانيت.
من الناحية النسيجية يؤدي اعطاء الفينوباربيتال الى تكاثر الخلايا الكبدية الشديد (تضخم بشكل الخلايا وتضخم بحجم الخلايا)، وسيتوبلازم محبب زجاجي، بينما ينتج عن مستخلص بذور براسيكا تحسنًا في التغيرات النسيجية المرضية التي تشمل صورة انتشار خفيف لتكاثر الخلايا وتصبغ السيتوبلازم بصبغة اليوزين. بالمقارنة مع مجموعة الفينوباربيتال، تسبب علاج المجموعة المختلطة ببذور براسيكا نيجرا لمدة 14 يومًا والفينوباربيتال لمدة 7 أيام في انخفاض كبير في وزن الكبد النسبي وألانين أمينوترانسفيراز. يحتوي مستخلص بذور الخردل الأسود على ايزوثايوسايانيت كمركب رئيسي أظهر تأثيرًا مضادًا للتكاثر على نسيج الكبد، مما يعني أنه قد يكون له تأثير واعد في تقليل مخاطر الإصابة بسرطان الكبد.

الكلمات المفتاحية: سرطان الكبد, الفينوباربيتال, البراسيكا نايجرا

Introduction

Hepatocellular proliferation (HCC) considered the most widespread form of primary liver cancer, most commonly occurs in patients with chronic liver disorders, like cirrhosis, infection by hepatitis C or B virus [1]. Cell proliferation is a main requirement for the beginning, promotion, and advancement of hepatocellular carcinoma (HCC) [2] Phenobarbital considered as a potent non-genotoxic type of tumor promoter agents in the rodent liver. Phenobarbital has been intensively researched as an epigenetic liver tumor promoter in rodents. PB has demonstrable effects on hepatocytes at exposures that cause rodent liver tumors: PB inhibits cell-to-cell communication; PB activates enzymes, including P450 cytochromes; PB stimulates hepatocyte proliferation and inhibits apoptosis in neoplastic foci [3].

In several years have been seeing that high consuming of Brassicaceae vegetables minimize the risk of a large number of cancers, like lung [4], gastrointestinal tract [5,6], prostate [7]) and bladder cancers [8], [9,10].

Materials and Methods

Chemicals and reagents:

Chloroform was purchased from British drug house, UK. Ethanol 99 from Alpha chemo, India. Formaldehyde PanReac Applichem, USA. Na2HPO4 and NaH2PO4 from Fluca, Germany. Eosin from Thomas Baker, India. Hematoxylin from Gourilabs, France. Paraffin wax and Xylene from Scharlau, Spain. Phenobarbital ampoule (200mg/ml) supplied from Samarth life sciences PVT.LTD, India. Alanine aminotransferase enzyme (ALT) and Aspartate aminotransferase enzyme (AST) kits from Bioassay Technology Laboratory.

The plant:

Brassica nigra seeds were obtained from the local market in Baghdad/Alshorga in September 2020, and were successfully identified by a professional plant taxonomist in the Department of Pharmacognosy/Collage of Pharmacy/AL-Mustansiriyah University, and a professional plant taxonomist in the Collage of Science / Baghdad University. The seeds were then stored in a dark place for a few days prior to the extraction process.

Animals: Twenty eight, non-previously treated male Swiss albino mice aged (8weeks) their weight (25 ±5) gm provided from Iraqi Center For Cancer Research were retained in well ventilated place with woodchip bedding with well-marked by their tails for identification and were maintained under standard conditions of relative humidity (70 ± 5%), Temperature (25 ± 2°C), and a 12-hour light-dark cycle with free access to water and food (pellets) in the animal house of the Collage of Pharmacy/Al-Mustansiriyah University.

Extract preparation: *Brassica nigra* seeds (2000g) were air dried and finely grinded by electrical mill. These seeds were subjected to extraction by reflux extraction processes. Firstly, the powdered seeds had been immersed with absolute ethanol (99%) and water at 70:30 percent in round reflux apparatus at a temperature range from 50-55 ^{0} c for 1hr, after that the seeds residues were filtered using filter paper to get clean crude extract, Finally, the filtrate undergo evaporation by a rotary evaporator and further air drying till a semisolid paste was obtained. The extract that obtained at weight 140gm was labelled and preserved in a sealed glass container in refrigerator at 4 ^{0}c till the time of experiment. The yield of extract was found to be 7% (w/w) according to the following equation [11].

The yield of extract = Weight of material obtained /Weight of starting material × 100.

Phytochemical screening: Phytochemical screening of hydro alcoholic extract of *Brassica nigra* using standard procedure for detection the presence of glycosides, alkaloids, flavonoids, saponine, tannin and phenols [12,13].

Gas chromatography-mass spectrometry (GC-MS analysis): This technique commonly used for both qualitative and quantitative analysis of a sample containing a variety of organic compounds[14].The GC-MS technique was used in the current study to recognize the phytoconstituents present in the *Brassica nigra* extract. This work was done in the Ministry of Science and Technology, Environment and Water Department. The GC-MS was carried out using a Schimadzu (QP2010) PLUS system. The column used in GC-MS system was (optima -5ms, Medium non polar 30m length, 0.25 um thickness). The chemical compounds from the *Brassica nigra* extract were known by comparing between the retention times of the obtained chromatographic peaks.

Animal grouping: Twenty-eight male Swiss albino mice were randomly divided into four groups (n=7for each): First group (Control group): received normal saline by oral gavage for one week and continue with normal saline intrapertonially for the second week. Second group (Phenobarbital group): received normal saline by oral gavage for one week, then intrapertonial phenobarbital at 75mg/kg /day for the second week. Third group (Extract alone group): received the extract at dose 800mg/kg/day by oral gavage for one week, then continue with extract at dose 800mg/kg/day by oral gavage for the second week. Fourth group (Combination group): received the *Brassica nigra* extract at dose 800mg/kg/day by oral gavage for one week, then continued with *Brassica nigra* extract at dose 800mg/kg/day by oral gavage with intrapertonial phenobarbital at 75mg/kg /day for the second week.

Clinical observations: All animals were observed daily for any difference in food and water intake, and any signs of abnormality during the experiment period. Total body weight was measured for all animals group every week using sensitive electrical balance. The percent of body weight changes, relative organ weight was calculated according to equations [15] as follow:

% Of body weight change=Final Weight-Initial weight/ Final weight x 100

% Of relative organ weight= Organ weight / weight of mice at scarifying day x100

Serum collection: After anaesthetization of animals using chloroform (inhalation), blood was collected directly from the heart using (5 CC syringe). The blood samples were placed in gel tube for 20 minute at room temperature then centrifuged at (2000-3000 RPM) for 15 minute to separate the serum. Serum samples transferred in Eppendorf tubes and freezed at (-20) till assay for hepatic aminotransferases, urea and creatinine levels.

Liver harvest: After the ending of treatment period, animals were euthanized by placed in a container that contained the chloroform. To ensure circulation, the chemical was soaked in cotton wool and placed in the container for 5 minutes before to exposure and liver tissues were collected surgically.

Histopathological analysis: The liver tissues transferred in buffered formalin (100 ml of 40% formalin+900 ml distilled water+4 gm of sodium di-hydrogen phosphate+6.5 gm of sodium phosphate dibasic) After fixation, the tissue samples were immersed in ascending grades of ethanol to avoid excessive tissue hardening to prepare the sample for the next step as a following procedure (Eighty percent ethanol for 15 minutes, Ninety five percent ethanol for 15 minutes), Absolute ethanol (99.9%) for 15 minutes. Chloroform for 15 minutes. Then Paraffin wax was molted at 60 ˚C for 15 minutes [17]. Finally, samples were labeled and inserted in paraffin wax as a block that prepared to histopathological analysis by a professional pathologist. Liver injury was graded depending on specific scoring system [18] which categorized according to hepatocyte size, cytoplasmic granularity and inflammation that included: (1) No abnormalities, (2) Mildly abnormalities, (3): Moderately abnormalities.

Liver enzymes measurement: Measurement of the liver enzyme aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were done in Collage of Pharmacy /AL-Mustansiriyah University according to manufacturer procedure using sandwich enzyme- linked immune sorbent assay (ELISA) technique [16].

Statistical analysis: The statistical analysis of this prospective study was performed with the statistical package for social sciences (SPSS) version 24.0 and Microsoft Excel 2020. Chi-square, Kruskal Wallis and Mann Whitney tests were used to describe the association of categorical data. Numerical data were described as mean and standard error. Analysis of variance (ANOVA) was used for comparison among more than two groups. *P- Value* <0.05 considered as a significant difference.

Results

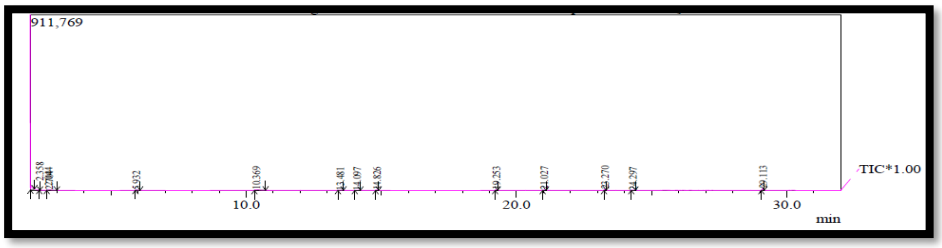
Phytochemical screening tests: In this study, the yield percentage of the *Brassica nigra* seeds extract was 7%, since 140 gm was obtained from 2000gm of *Brassica nigra* seeds. Qualitative phytochemical studies were performed on the hydro alcoholic extract of *Brassica nigra* seeds to determine the presence of various phytochemical constituents. The results showed the presence of glycosides, alkaloids, terpenoids, flavonoids, phenols, tannin, saponin and cumarin, table (1).

Table (1): Preliminary tests of hydro alcoholic extract of *Brassica nigra* seeds.

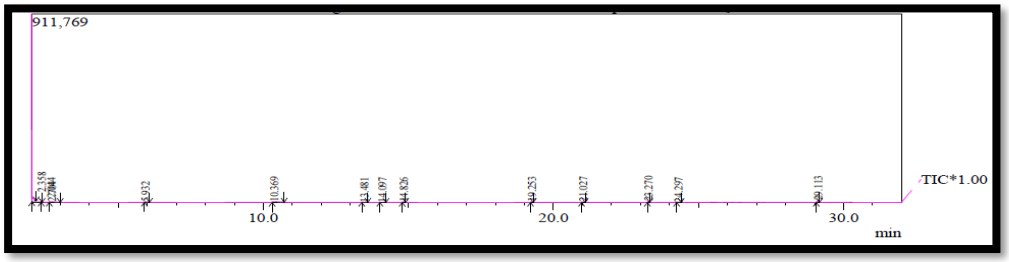
Chemical	Result	Chemical	Result
Alkaloids	+	Terpenoids	+
Flavonoids	+	Glycosides	+
Saponin	+	Cumarin	+
Tannins, Phenols	++		

(+): present, (++): highly present

Gas chromatography-mass spectrometry (GC-MS) analysis: The most important recognized chemicals were isothiocynates like allyl and butyl isothiocynate. Figures (1) showed the peaks of these chemicals.



(A)



(B)

Figure (1): (A) GC-MS analysis results of *Brassica nigra* hydro alcoholic seeds extract. (B) Isothiocynate compound detected by GC-MS.

Effect of hydro alcoholic seeds extract of *brassica nigra* on mice clinical signs, body weight and relative organs weight

Treatment of mice with phenobarbital, *Brassica nigra* extract or their combination didn't show any clinical signs of toxicity during the experiment period while decrease in physical activity and change in mice behavior was observed in phenobarbital and combination groups that is related to sedative activity of phenobarbital. Regarding animal weight, extract group showed a significant (p<0.05) elevation in mean level of body weight when compared with control group, phenobarbital group and combination group, meanwhile there was no-significant (p>0.05) difference in mean values of this parameter among these groups, as demonstrated in figure (2).

Regarding to the relative liver weight, phenobarbital caused significant (p<0.05) elevation in this parameter when compared with control, extract and combination group, while there was no significant difference among control, extract and combination groups, as demonstrated figure (2).

Considering relative kidney weight, there was no-significant (p>0.05) difference in mean values of this parameter among all studied groups, as shown in figure (2).

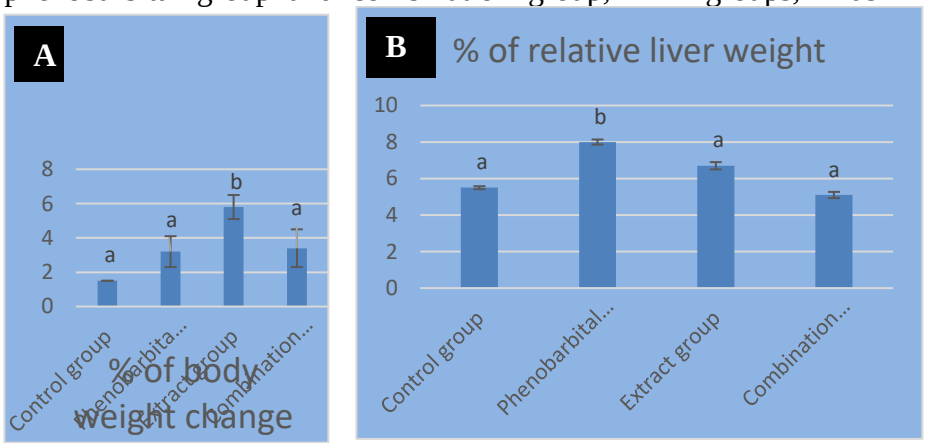
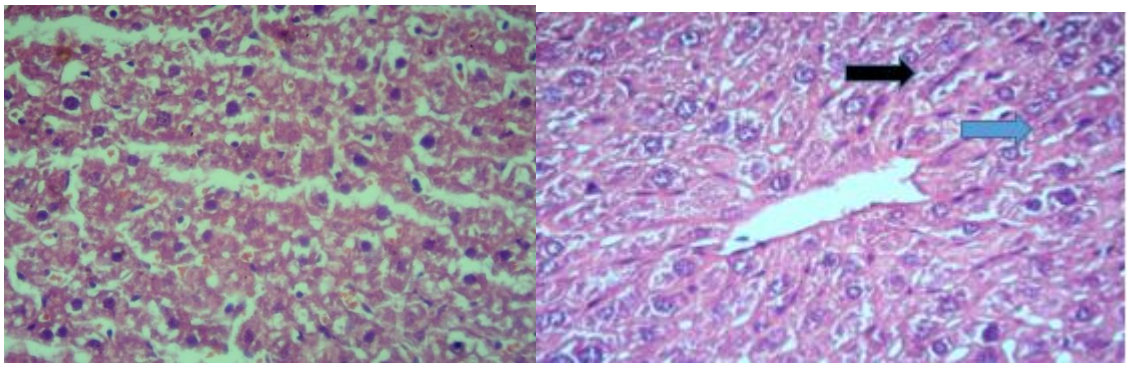


Figure (2): Effect of hydro alcoholic *Brassica* seeds extract on A: body weight, B: relative liver weight.

Data were expressed as means ± SEM. Different superscript small letters (a,b) represent significant differences among the examined groups**.** *P- value* <0.05 indicate a significant difference.

Effect of hydro alcoholic *Brassica nigra* seeds extract on mice liver histology

Light microscopical observations of the liver tissues showed that control group exhibited apparently normal number and size of hepatocytes with eosinophilic cytoplasm, as shown in figure (3-A), while phenobarbital induced severe hepatocellular proliferation (hyperplasia and hypertrophy), glass ground cytoplasm and pericentral sinusoidal dilatation, when compared with other studied groups, as shown in figure(3-B).However, microscopical observation of extract only group showed apparently normal hepatocyte picture and cytoplasmic appearance with no obvious pathological changes, as shown in figure (3-C). Regarding the combination group, these mice showed mild scattered proliferation picture (mild hyperplasia and hypertrophy of hepatocyte) in comparism with other groups, as shown in figure (3-D).



(A)

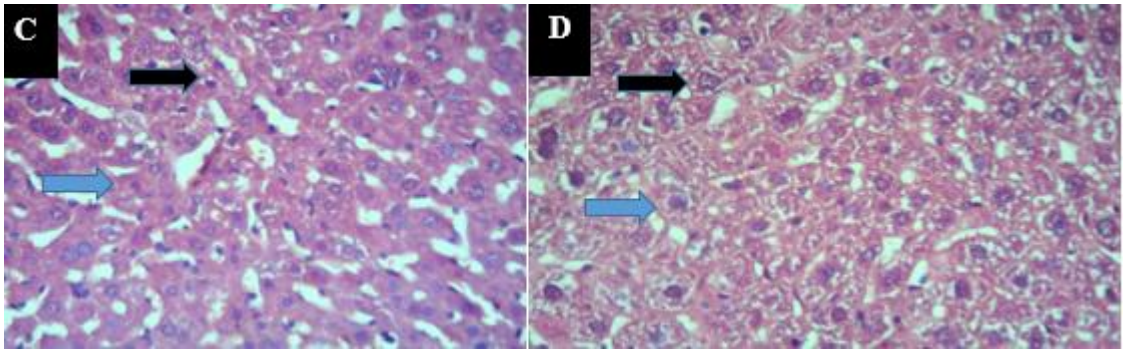


Figure (3): Section of the liver mice A: control group, B: phenobarbital group, C: extract group D: combination group. (Black arrow represent nuclei, blue arrow represent hepatocyte). Hemotoxylin &eosin stain (40x).

In the present study, histopathological scoring system was used for determination of hepatocellular proliferation graded, as score value high as hepatocellular damage high. Mice exposed to phenobarbital showed higher score with significant difference in means values of hepatocyte size, cytoplasmic granularity compared with other groups with. Meanwhile, mice that received *Brassica nigra* extract showed less pathological changes of liver an approach to that of control, table (2).

Table (2): Effect of hydro alcoholic *Brassica nigra* seeds extract on histopathological scoring system.

Groups	n	Hepatocyte size	Cytoplasmic granularity	inflammation
Control group	7	7.86[a]	7.86 [a]	12.07 [a]
Phenobarbital group	7	24.57[b]	24.57 [b]	16.29 [a]
Extract group	7	9.21[a]	9.21[a]	11.71 [a]
Combination group	7	16.36[c]	16.36[c]	17.93 [a]

Data are reported as means rank. Differant superscript small letters (a) indicate significant differences among the studied groups. *P- Value* <0.05 considered as a significant difference.

Effect of hydro alcoholic seeds extract of *Brassica nigra on* serum activities of liver enzymes

It is noted from figure (4) the mean serum levels of AST showed a non-significant difference (p>0.05) among all of the studied groups. Meanwhile, mice with phenobarbital caused a significant elevation (P < 0.05) in the mean level of serum ALT, compared with the control, extract, and combination group, as shown in figure (4).

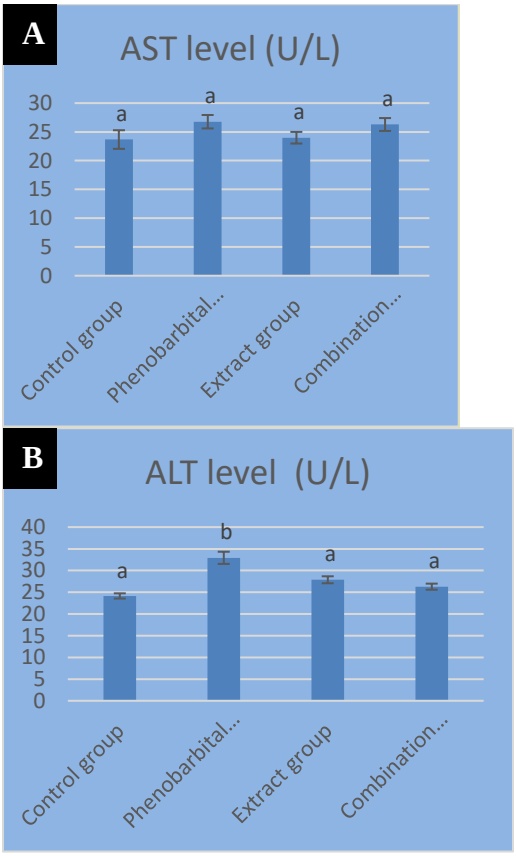


Figure (4): Effect of hydro alcoholic *Brassica nigra* seeds extract on ALT liver enzyme activity, A: AST level, B: ALT level. Data were expressed as means ± SEM. Different superscript small letters (a,b) represent significant differences among the examined groups .*P- value* <0.05 indicate a significant difference.

DISCUSSION

Hepatocellular carcinoma (HCC) still the most widespread form of primary liver cancer and a major cause of cancer-correlated death around the world [(19)]. By 2030, the global burden of HCC mortality is expected to reach one million death per year [(20)].It has been established that a high intake of Brassica vegetables is linked to a lower risk of cancer, this link clearly appears in lung, stomach, colon, rectal and prostate cancer [(21,22,23)]. In the present study, the percent of yield from Brassica nigra seed was 7%, using water- ethanol solvent system, according to the reflex extraction method [(24,25)].This result was higher than others which were conducted by Upwar N. (2011)[(26)] who used methanol as solvent (4.3% w/w) and Anand P. (2014)[(27)] that was 5.18 % (w/w) using DW as a solvent and Basha S. (2015)[(28)] that was 1.4% (w/w) using DW and 6.52 % (w/w) using methanol.

This finding largely related to the extraction process, since the isolation of bioactive chemicals from plant materials using different temperatures during extraction technique is very important, where the findings of previous research revealed that increasing temperature can increase the yield of extraction for *Brassica nigra* and this support the current results [(29)]. The present study aimed to investigate the phytochemicals of *Brassica nigra* seeds extract. The results showed that the plant contains flavoinds, alkaloids, terpenes, saponines, tannins and glycosides, these classes of chemical compounds were same to those of previous studies conducted by Uddin MS. (2020) [30], Danlami U. (2016) [(31)], and Basha S. (2015) [28]. In the current study, GC-MS analysis confirmed the presence of isothiocyanates represented by 3-butenylisothiocyanate and methyl isocyanate.The present study come in accordance with Sharma A. (2018) who detect the same isothiocyanate compounds

from *Brassica juncea* (a plant with same family of *Brassica nigra*), since isothiocyanate present in abundance within Brassicaceae family [32].

The mean body weight of all treatment groups gradually increased over the observation period. According to OECD (Organization for Economic Co-operation and Development) guideline, the weight variations in mice are acceptable if they did not exceed ±20% SD of the mean weight [33].

In this study, significant increase in body weight was observed in extract group compared with other groups, the result agreed with Arogundade et al., study (2017) since mustard seeds are thought to have appetite-stimulating activities that could likely cause increment in animal body weight. It's possible that the *Brassica nigra* seeds extracts activate the hypothalamic lateral nuclei and appetite to the point where the animals eat not because they're hungry, but because there's food available, which could explain why the *Brassica nigra* only group gained more weight than the other groups [34]. A study done by Kumar *et al.*, (2013) also found a weight gain in animal groups treated with *Brassica nigra* when compared with that of control, the study evaluated antioxidant action of *Brassica nigra* oil in experimental rats [35].

Relative liver weight was the first parameter employed in this study to assess the effect of *Brassica nigra* extract on liver organ. The ratio of liver weight to body weight is a useful indicator of the healthy state of hepatic tissues [36]. There was a positive association between liver weight and hepatocellular hyperplasia or carcinoma in mice that were previously studied by Elcombe *et al.*, (2002) [37] and Maronpot R.(2010) [38]. Maronpot R. also reported that a subcapsular necrosis of hepatocytes can occurs when there is an extensive increase in liver size that may contributes to the mechanical impacts of compression [38].After seven days of phenobarbital (PB) exposure, the mice liver organs were enlarged, as demonstrated by statistically significant elevation in mean liver weight and in liver to total weight ratio in comparison with other groups, this elevation is due to enzyme induction and hepatocyte proliferation [38].Wei *et al.*, (2000) reported that the increment in liver weight in response to PB is correlated to CAR-activation [39].

The result of the current experiment regarding liver weight was in agreement with that of Nesnow S. (2009) [40], Jones HB. (2009) ([41], Waterman CL. (2010) [42] and Geter DR. (2014) studies [43]. The ratio of relative liver mice weight used as experimental animals was 3-5% BW (2-3 g) [44]. In this study, the increase in relative liver weight in phenobarbital group considered significant adverse effect on liver tissue since it was accombinaned within significant increase in alanine aminotransferase (ALT) level, small elevation in aspartate aminotransferase (AST) level and significant pathological changes that discussed later [45]. One the other hand, the current research confirmed a decrement in liver weight within combination group when compared with phenobarbital group, this was the first sign giving impression that the extract could have anti-proliferative effect on hepatocytes. The result reported by Anand P. (2009) study indicated that the diabetic rats treated with *Brassica nigra* extract exhibited improvement in liver weight, where the researcher found that the extract can restore the liver weight near normal level compared with diabetic group not exposed to *Brassica nigra* extract [27]. Similarly, Jie M. (2014) also found a liver weight reduction effect of sinigrin (an essential component of *Brassica nigra* seeds) in rats exposed to hepatotoxic and carcinogenic substances and this support

the results of the present study [36]. These results suggest a protective effect of *Brassica nigra* seeds extract on liver organ, may be due to the synergistic effect of phytochemicals in this plant since it contains phenols, terpenes, glycosides and isothiocyanates, all of these compounds are reported to have antioxidant effect on liver tissues [46].

In the present study, liver proliferation that achieved by phenobarbital administration resulted in hepatocyte hypertrophy. Histological analysis reported that proliferation was effectively induced by phenobarbital since high doses of phenobarbital could cause marked hyperplasia and hypertrophy of hepatocyte and alteration in cytoplasmic characteristic (ground-glass appearance), a condition known as "liver proliferation", as reported by Gaskill CL. (2005) [47].Hepatocellular hypertrophy associated with phenobarbital administration considered a characteristic of enzyme induction, since there is an elevation of protein synthesis and increase in the number of cytoplasmic organelles [38]. Also, hepatocyte hypertrophy linked well with increment in the relative liver weight. The ground glass morphologic characteristics of hepatocytes under phenobarbital therapy may explained by the smooth endoplasmic reticulum (SER) proliferation as a consequence of microsomal enzyme activation induced by PB [48].

Interestingly, the results of the current study illustrated that there were distinct histological alterations between phenobarbital and the combination group of mice, since pretreatment with seeds extract of *Brassica nigra* protected the liver from the effect of phenobarbital. This finding was similar to that of Rajamurugan *et al.,* (2011) and Rajamurugan *et al.* (2012) studies which were described that the protective effect can be related to the presence of hepatoprotectants such linoleic acid, triterpenoids and also due to various antioxidant compounds that were found in *Brassica nigra* seeds [49,50] .Also, histopathological investigations achieved by Ahn M. (2016) on rats with allylisothiocynate (AITC) and carbon tetrachloride (CCl4) taken together have delivered evidence supporting the protective effects of AITC on CCL4 induced liver injury [51], so the synergistic effect of constituents within *Brassica nigra* extract is responsible for the observed effect and support the current finding.In the current study, phenobarbital as a model of hepatocellular proliferation, significantly increase activity of serum liver enzyme alanine aminotransferase (ALT), which was expected since phenobarbital is an enzyme inducer agent. This result was in line with Geter DR. (2014) [34] and Radwan SA. (2000) [52], but not with Mansour *et al.* (1995) [53], may be due to dose effect. Unexpectedly, aspartate aminotransferase (AST) level was not significantly differed among all studied groups, this was disagreed with Radwan SA. (2000) [52], while agree with Müller. PB (2000) finding who demonstrated that serum AST level not influenced by the enzyme-inducing action of phenobarbital in contrast to ALT, since there is different susceptibility of AST and ALT to enzyme induction [48].

However, the level of ALT showed significant decrease in combination group in compared to phenobarbital group. These results are in consistent with the finding of Rajamurugan *et al.,* (2011) study which evaluated *Brassica nigra* extract against liver damage [49].

The present experiment matched with Asaad NK. (2021) study which observed that treatment with seeds extract of *Brassica nigra* after cadmium chloride compound restored the serum level of hepatic enzymes, as indicator of the protective effect of *Brassica nigra* extract against cadmium-

liver toxicity, which may be attributed to the high levels of phenols and carotenoids [54].

The current research also agreed with Jie M. (2014) study which reported a preferable effect of sinigrin in reducing ALT level which was elevated by hepatotoxic carcinogens (36). In addition, Ahn .M (2016) proved that rats pretreated with allyl isothiocynate (AITC) orally cause significant decline in ALT level which was raised after exposure to CCL4 as hepatotoxic compound, and this finding support that of the current research (51). So, the proposed synergistic effect of isothiocynte, phenols, terpenoids and vitamins present in the *Brassica nigra* seeds extract may responsible for this protective effect.

As a future work, the following points can be suggested: The precise active phytochemicals of *Brassica nigra* that responsible for the observed effects are unknown, so fractionation of the extract, isolation and purification of active constituents is essential. Evaluate the in vitro and in vivo anticancer effect of the *Brassica nigra* extract on different cells and tissues using tumor-initiator agents, and try to understand the molecular mechanism and exact signaling pathway. Further researches may require to ensure the safety and tolerability of *Brassica nigra* extracts.

Conclusions

From the result of the present study one can conclude the followings: reflux extraction method using water: ethanol solvent system gave acceptable percent of yield and the major active phytochemicals may be represented by isothiocyanate, depending on GC-MS analysis. *Brassica nigra* extract showed antiproliferative effect estimated by liver weight measurement and histopathological changes. Brassica nigra seeds extract showed no toxic effect of liver tissue and can be have a role in minimizing liver injury.

Acknowledgement

The authors would like to thank Mustansiriyah University (www.uomustansiriyah.edu.iq), Baghdad, Iraq, for its support in the present work.

Referances

1- Issues CC. Current Clinical Issues Liver Cancer on the Rise TO. 2005;1029–32.
2- Maronpot RR. Biological basis of differential susceptibility to hepatocarcinogenesis among mouse strains. J Toxicol Pathol. 2009;22(1):11–33.
3- Whysner J, Ross PM, Williams GM. Phenobarbital mechanistic data and risk assessment: Enzyme induction, enhanced cell proliferation, and tumor promotion. Pharmacol Ther. 1996;71(1–2):153–91.
4- Müller PB, Taboada J, Hosgood G, Partington BP, VanSteenhouse JL, Taylor HW, et al. Effects of long-term phenobarbital treatment on the liver in dogs. J Vet Intern Med. 2000;14(2):165–71.
5- Smith TK, Lund EK, Clarke RG, Bennett RN, Johnson IT. Effects of Brussels sprout juice on the cell cycle and adhesion of human colorectal carcinoma cells (HT29) in vitro. J Agric Food Chem. 2005;53(10):3895–901.
6- Van Poppel G, Verhoeven DTH, Verhagen H, Goldbohm RA. Brassica vegetables and cancer prevention: Epidemiology and mechanisms. Adv Exp Med Biol. 2000;472:159–68.
7- Giovannucci E, Rimm EB, Liu Y, Stampfer MJ, Willett WC. A Prospective Study of Cruciferous Vegetables and Prostate Cancer. Cancer Epidemiol Biomarkers Prev. 2003;12(12):1403–9.

8- Abbaoui B, Lucas CR, Riedl KM, Clinton SK, Mortazavi A. Cruciferous Vegetables, Isothiocyanates, and Bladder Cancer Prevention. Mol Nutr Food Res. 2018;62(18):1–14.
9- Zhao H, Lin J, Grossman HB, Hernandez LM, Dinney CP, Wu X. Dietary isothiocyanates, GSTM1, GSTT1, NAT2 polymorphisms and bladder cancer risk. Int J Cancer. 2007;120(10):2208–13.
10- Mastuo T, Miyata Y, Yuno T, Mukae Y, Otsubo A, Mitsunari K, et al. Molecular mechanisms of the anti-cancer effects of isothiocyanates from cruciferous vegetables in bladder cancer. Molecules. 2020;25(3).
11- Omoja V, Ihedioha T, Eke G, PeterAjuzie I, Okezie S. Evaluation of the acute toxicity, phytochemical constituents and anti - ulcer properties of methanolic leaf extract of Annona muricata in mice. J Intercult Ethnopharmacol. 2014;3(1):37.
12- Evans W.C. Trease and Evan's Pharmacognosy. Haroourt Publishers,London, ISBN: 0702026174, 336-337.
13- Harborne J.B. Phytochemicsal methods: A Guide to Modern Techniques of Plant Analysis. London, Chapman and Hall, 2007; ISBN: 81-8128-410-4, 1-34.
14- Khalaf HA, Mahdi MF, Abaas IS. Preliminary Phytochemical and GC-MS analysis of chemical constituents of Iraqi Plantago lanceoleta L. Al-Mustansiriyah Journal of Pharmaceutical Sciences (AJPS) (2018); 18.2: 114-121.
15- Souza BA. Manual de ensaios toxicológicos in vivo.Manual de ensaios toxicológicos in vivo. 1994. 122-122.
16- Bioassay Technology Labrotary kit protocol available from : (www.bt-laboratory.com | 1008 Junjiang Inter. Bldg. 228 Ningguo Rd. Yangpu Dist. Shanghai. China Tel: 86 21 65109711 | Fax: 86 21 65109711 816 | E-mail: save@bt-laboratory.com)
17- Anthony L. Junqueira's Basic Histology text and atlas. Edition. McGraw-Hill Education. 2016
18- Gaskill, C. L., et al. "Liver histopathology and liver and serum alanine aminotransferase and alkaline phosphatase activities in epileptic dogs receiving phenobarbital." Veterinary Pathology 42.2 (2005): 147-160
19- Balogh J, Iii DV, Gordon S, Li X, Ghobrial RM, Jr HPM. Jhc-3-041. J Hepatocell Carcinoma [Internet]. 2016;Volume 3:41–53. Available from: https://www.dovepress.com/hepatocellular-carcinoma-a-review-peer-reviewed-article-JHC.
20- Kim E, Viatour P. Hepatocellular carcinoma: old friends and new tricks. Exp Mol Med [Internet]. 2020;52(12):1898–907. Available from: http://dx.doi.org/10.1038/s12276-020-00527-1
21- Ahmed AG, Hussein UK, Ahmed AE, Kim KM, Mahmoud HM, Hammouda O, et al. Mustard Seed (Brassica nigra) Extract Exhibits Antiproliferative Effect against Human Lung Cancer Cells through Differential Regulation of Apoptosis, Cell Cycle, Migration, and Invasion. Molecules 2020;25(9):2069. Available from: http://dx.doi.org/10.3390/molecules25092069
22- Verhoeven DTH, Goldbohm RA, Van Poppel G, Verhagen H, Van Den Brandt PA. Epidemiological studies on Brassica vegetables and cancer risk. Cancer Epidemiol Biomarkers Prev. 1996;5(9):733–48.
23- Kristal AR. Brassica vegetables and prostate cancer risk: A review of the epidemiologic evidence. Pharm Biol. 2002;40(SUPPL.):55–8.

24- Nandini T, Ramya Sree D, Janbee S, Siva Sai Kiran K, Raveendra Babu G. Study of immunomodulatory effect of seeds of Brassica nigra and Cuminum cyminum in albino rats. Int J Pharm Clin Res. 2016;8(1):10–4.
25- Ahmed, Asmaa Gamal, et al. "Mustard seed (Brassica nigra) extract exhibits antiproliferative effect against human lung cancer cells through differential regulation of apoptosis, cell cycle, migration, and invasion." Molecules 25.9 (2020): 2069.
26- Upwar N, Patel R , Waseem N , Mahobia N. In vitro anthelmintic activity of Brassica nigra Linn. seeds. (2011).
27- Anand P, Murali YK, Tandon V, Murthy PS, Chandra R. Insulinotropic effect of aqueous extract of Brassica nigra improves glucose homeostasis in streptozotocin induced diabetic rats. Exp Clin Endocrinol Diabetes. 2009;117(6):251–6.
28- Basha SN, Rekha R, Saleh S, Yemane S. Evaluation of invitro anthelmintic activities of Brassica nigra, Ocimum basilicum and Rumex abyssinicus. Pharmacogn J. 2011;3(20):88–92.
29- Radha Krishnan K, Azhagu Saravana Babu P, Babuskin S, Sivarajan M, Sukumar M. Modeling the Kinetics of Antioxidant Extraction from Origanum vulgare and Brassica nigra. Chem Eng Commun. 2015;202(12):1577–85.
30- Uddin MS, Millat MS, Islam MS, Hussain MS, Uddin MG, Siddiqui SA, et al. Exploration of in vitro thrombolytic, anthelminthic, cytotoxic and in vivo anxiolytic potentials with phytochemical screening of flowers of Brassica nigra. Futur J Pharm Sci. 2020;6(1).
31- Danlami U. Phytochemical, Nutritional and Antimicrobial Evaluations of the Aqueous Extract of Brassica Nigra (Brassicaceae) Seeds. 2016; 4(4):161.
32- Sharma A, Rai PK, Prasad S. GC–MS detection and determination of major volatile compounds in Brassica juncea L. leaves and seeds. Microchem J [Internet]. 2018;138(2017):488–93. Available from: https://doi.org/10.1016/j.microc.2018.01.015
33- Organisation for Econommic Cooperation and Development,OECD Guidline for the testingof chemicals.Test guidline 423, acute oral toxicity –acute toxic class method.http://www.oecdilibrary.org.
34- Arogundade TT, Enaibe BU, Adigun OO, Adigun FM, Gbadamosi IT. Dose-dependent effect of black mustard seeds (Brassica nigra) extract on the prefrontal cortex of adult Wistar rats. Anatomy. 2017;11(3):107–14.
35- Kumar M, Sharma S, Vasudeva N. In vivo assessment of antihyperglycemic and antioxidant activity from oil of seeds of Brassica nigra in streptozotocin induced diabetic rats. Adv Pharm Bull. 2013;3(2):359–65.
36- Jie M, Cheung WM, Yu V, Zhou Y, Tong PH, Ho JW. Anti-proliferative activities of sinigrin on carcinogen-induced hepatotoxicity in rats. PLoS One. 2014 Oct 20;9(10):e110145. doi: 10.1371/journal.pone.0110145. PMID: 25329483; PMCID: PMC4203766.
37- Elcombe CR, Odum J, Foster JR, Stone S, Hasmall S, Soames AR, et al. Prediction of rodent nongenotoxic carcinogenesis: Evaluation to biochemical and tissue changes in rodents following exposure to nine nongenotoxic NTP carcinogens. Environ Health Perspect. 2002;110(4):363–75.
38- Maronpot RR, Yoshizawa K, Nyska A, Harada T, Flake G, Mueller G, et al.

Liver enlargement - STP regulatory policy papers: Hepatic enzyme induction: Histopathology. Toxicol Pathol. 2010;38(5):776–95.

39- Wei, P., Zhang, J., Egan-Hafley, M. et al. The nuclear receptor CAR mediates specific xenobiotic induction of drug metabolism. Nature 2000; 407, 920–923 https://doi.org/10.1038/35038112

40- Nesnow S, Ward W, Moore T, Ren H, Hester SD. Discrimination of tumorigenic triazole conazoles from phenobarbital by transcriptional analyses of mouse liver gene expression. Toxicol Sci. 2009;110(1):68–83.

41- Jones HB, Orton TC, Lake BG. Effect of chronic phenobarbitone administration on liver tumour formation in the C57BL/10J mouse. Food Chem Toxicol. 2009 Jun;47(6):1333-40. doi: 10.1016/j.fct.2009.03.014. Epub 2009 Mar 17. PMID: 19298838.

42- Waterman CL, Currie RA, Cottrell LA, Dow J, Wright J, Waterfield CJ, et al. An integrated functional genomic study of acute phenobarbital exposure in the rat. BMC Genomics. 2010;11(1):1–17.

43- Geter DR, Bhat VS, Bhaskar Gollapudi B, Sura R, Hester SD. Dose-response modeling of early molecular and cellular key events in the CAR-mediated hepatocarcinogenesis pathway. Toxicol Sci. 2014;138(2):425–45.

44- KILIMCI, Figen SEVIL. Rabbit liver lobes: an anatomical study of experimental surgical approaches. Erciyes Üniversitesi Veteriner Fakültesi Dergisi, 2020, 17.2: 103-108.

45- Health H, Meeting J, Panel FAO, Residues P, Core WHO, Group A, et al. ”Interpretation of liver effects” Provided by UK. 2018;1–4.

46- Melega S, Canistro D, De Nicola GR, Lazzeri L, Sapone A, Paolini M. Protective effect of Tuscan black cabbage sprout extract against serum lipid increase and perturbations of liver antioxidant and detoxifying enzymes in rats fed a high-fat diet. Br J Nutr. 2013;110(6):988–97.

47- Gaskill CL, Miller LM, Mattoon JS, Hoffmann WE, Burton SA, Gelens HCJ, et al. Liver histopathology and liver and serum alanine aminotransferase and alkaline phosphatase activities in epileptic dogs receiving phenobarbital. Vet Pathol. 2005;42(2):147–60.

48- Müller PB, Taboada J, Hosgood G, Partington BP, VanSteenhouse JL, Taylor HW, et al. Effects of long-term phenobarbital treatment on the liver in dogs. J Vet Intern Med. 2000;14(2):165–71.

49- Rajamurugan R, Selvaganabathy N, Kumaravel S, Ramamurthy C,Sujatha V, Thirunavukkarasu C. Polyphenol contents and antioxidant activity of Brassica nigra (L.) Koch. leaf extract. NatProd Res.(2011).

50- Rajamurugan R, Selvaganabathy N, Kumaravel S, Ramamurthy C, Sujatha V, Thirunavukkarasu C. Polyphenol contents and antioxidant activity of Brassica nigra (L.) Koch. leaf extract. Nat Prod Res. 2012;26(23):2208–10

51- Ahn M, Kim J, Bang H, Moon J, Kim GO, Shin T. Hepatoprotective effects of allyl isothiocyanate against carbon tetrachloride-induced hepatotoxicity in rat. Chem Biol Interact. 2016;254:102–8. Available from: http://dx.doi.org/10.1016/j.cbi.2016.05.037

52- Radwan SA, El-wessemy AM, Sakr SM. Sodium Barbital Induced Biochemical , Histological and Histochemical Changes in the Liver of

Albino Mouse * Department of Biological Sciences , Faculty of Education , Ain Shams University , Cairo , Egypt . ** High Institution of Applied arts , 6 th October City Abstract. 2000;(1971).

53- Mansour A, Abdel-Raheem K, El-Mossallamy N, El-Yamany N , Mahmoud S .Impact of chronic administration of ethanol and/or phenobarb-itone and drug withdrawal on transaminases and proteins in blood and liver of albino rats. J. Egypt. Ger. Soc. Zool.1995;16 (A): 429-450.

54- Asaad NK, Razooqi QA, Mustafa MA. Toxicity of Cadmium Chloride on White Rats Liver and the Protective Role of Brassica Nigra Seed Extract. Indian J Forensic Med Toxicol. 2021;(January).