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Original Article

Effect of *Berberis vulgaris* fruit extract on alpha-fetoprotein gene expression and chemical carcinogen metabolizing enzymes activities in hepatocarcinogenesis rats

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Abstract

Objective: Cancer is the second cause of death worldwide, among them liver cancer is the fifth most important malignancy. The aim of this study was to investigate chemopreventive activity of *Berberis vulgaris* fruit extract in hepatocarcinogenesis *Sprague dawley* rats.

Materials and Methods: Fourty-four *Sprague dawley* rats (6-8 weeks old, 150-250 g) were divided into normal and cancerous. The rats in cancerous groups induced cancer by intraperitoneally injection of 200mg/kg diethylnitrosamine (DEN) follow by acetyllaminofluorene (0.02%AAF) dissolved in corn oil. Animals in each branch were randomly assigned to receive either no treatment or 25, 50 or 100 mg/kg of *Berberis vulgaris* fruit. The animals were sacrificed after 11 weeks of treatment and evaluated for liver enzyme activities. In addition, Reverse-transcription polymerase chain reaction was performed to identify the expression of genes encoding markers in hepatocytes (albumin, α-fetoprotein).

Results: The activities of GGT, GST and ALP between the Cancerous and Normal groups were significantly different ($P< 0.05$). In cancerous groups treated with *Berberis vulgaris*, the enzymes activities of GST, GGT and ALP were significantly lower than control group ($P< 0.05$). RT-PCR as a very sensitive method did not show gene expression of AFP in cancer groups treated with *Berberis vulgaris* fruit.

Conclusion : *Berberis vulgaris* fruit can reduce the activity of liver enzymes and inhibit the gene expression of AFP in rats during hepatocarcinogenesis. These results suggest the chemopreventive activity of *Berberis vulgaris* fruit against hepatocarcinogenesis in animal models.

Keywords : berberis vulgaris –alpha-fetoprotein- fruit extract -hepatocarcinogenesis

Introduction

Cancer is the second cause of death worldwide and it is estimated that 12.8% of the world population die due to cancer [1]. The number of new cases is increasing every year [2,3]. Hepatocarcinogenesis, hepatocellular carcinoma or liver cancer is one of the most prevalent and deadly cancers worldwide [4].

Fruits and vegetables account for a small part of our daily caloric intake; however, their benefits to heath surpass their caloric contribution. The contributory factors are due to the presence of vitamins and provitamins, such as ascorbic acid, tocopherols and carotenoids as well as a wide variety of phenolic substances [5].The principle function of antioxidants is delaying the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals, thus, they reduce oxidative damage to the human body [6,7].

The Solt & Farber protocol has been used to study the mechanism of modulatory factors of

hepatocarcinogenesis [8,9]. Diethyl nitrosamine (DEN) is a major carcinogen, which can induce liver cancer slowly with a single oral, intravenous or intraperitoneal administration [10]. It forms alkyl DNA adducts in the liver and induces hepatocellular carcinoma without cirrhosis through the development of putative preneoplastic condition, so-called enzyme-altered focal lesions [11,12]. Acetylaminofluorene (AAF) is an example of the classes of synthetic chemical carcinogens, which require metabolic activation. γ-glutamyl transpeptidase (GGT), GST and alkaline phosphatase (ALP) have been recognized as a positive marker for hepatocytes, which have undergone malignant transformation [13]. GGT is induced to high levels in many pre-neoplastic lesions (altered hepatic foci, AHF) at early stages of hepatocarcinogenesis (HC) in rodents [14-16]. The ubiquity of elevated GGT levels in many rodent and human hepatic and extrahepatic carcinomas [14-20] have led to the hypothesis that GGT provides a growth advantage to focal cells during carcinogenesis. Phase II enzymes such as glutathione-*S*-transferase (GST) and quinone reductase (QR) represent major detoxification pathway for conjugation and solubilization of diverse forms of xenobiotics rendering their excretion from the system [21]. ALP is an enzyme that transports metabolites across cell membranes. Liver and bone diseases are the most common causes of pathological elevation of ALP levels [22]. Knowledge of the pathophysiology of liver enzymes is an essential guide to understanding their alteration. The pattern of enzyme abnormality, interpreted in the context of the patient's characteristics, can aid in directing the subsequent diagnostic work-up [22]. The protein that would later be called alpha-fetoprotein (AFP) was first identified in human fetal sera in 1956. It became clear that AFP is a valuable marker in the differential diagnosis of HCC. An important contribution to this problem was made by extensive studies in West Africa and various other African regions [23].

Barberry (*Berberis vulgaris*) grows in Asia and Europe. Medicinal properties for all parts of the plant have been reported including tonic, antimicrobial, antiemetic, antipyretic, antipruritic and cholagogue actions, and it has been used in some cases like cholecystitis, cholelitiasis, jaundice, dysentery, leishmaniasis, malaria and gall stones [24]. The purpose of this study is to examine the antioxidant properties of *Berberis vulgaris* fruit extract on liver enzymes activities and alpha-fetoprotein gene expression in hepatocarcinogenesis rats.

Materials and Methods

Berberis vulgaris fruit was purchased from Iranian markets at Kuala Lumpur in Malaysia. Forty four female *Sprague dawley* rats were purchased from the animal colony unit, University Putra Malaysia, Serdang, Selangor.

Treatment of animals

Protocol of the rat hepatocarcinogenesis in this study was basically according to Solt and Farber method [10]. Forty four female *Sprague dawley* rats each initially weighing between 120-230 g (6-8 weeks old) were randomly distributed into eight groups, and were housed 3 rats in each cage for 11 weeks in a ventilated room temperature (22±2°C). Rat feed and water *ad libitum* were given to these rats daily. **N**ormal control group (NC), Normal group + *Berberis vulgaris* fruit extract (BFE)(25 mg/kg body weight/day)(NB_{25}), Normal group + BFE (50 mg/kg body weight/day) (NB_{50}), Normal group + BFE (100 mg/kg body weight/day)(NB_{100}), DEN/AAF control group (DAC), DAC group + BFE (DAB_{25}), DAC group + BFE (DAB_{50}), DAC group + BFE (DAB_{100}). The rats had free access to water. Each rat in NB (normal +*Berberis vulgaris* fruit extract) and DAB (DEN/AAF+*Berberis vulgaris* fruit extract) groups were given 25, 50 and 100 mg/kg body weight/day *Breberis vulgaris* fruit extract daily forcefeeding. Rats in DEN/AAF groups were intraperitoneally given a single injection of DEN(200mg/kg body weight) dissolved in corn oil at the beginning of experiment to initiate hepatocarcinogenesis followed by a recovery period of 2 weeks on a basal diet. The rats were then fed with 0.02% (w/w) AAF-treated rat chow for another 2 weeks without partial hepatectomy (selection pressure) to promote hepatocarcinogenesis. Treatment with 25, 50 and 100 mg/kg body weight/day *Breberis vulgaris* fruit extract was given by force feeding in DAB and NB groups. Rats in DEN/AAF groups were supplemented with 0.02% (w/w) AAF treated diet for two weeks after the second week. Except these two weeks, all rats were given basal diet. The rats

were sacrificed by decapitation under ether anesthesia at 11 weeks.

Cytosolic and microsomal fractions

Cytosolic and microsomal fractions of the livers were prepared based on the modified method of Speir and Wattenberg [25]. It was used for ALP and GST assay, while the pellet obtained contained the microsomal fraction was used for GGT assay.

Plant extract preparation

Berberis vulgaris fruit extract was prepared according to method of Motalleb *et al* [24].

Enzymes activities Assay

The activities of GST in the liver cytosol were assayed according to the method of Habig *et al* [26] using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate. The activity of GGT in liver microsomes was measured by standard method of Szasz [27] using γ-glutamyl-p-nitroanilide as substrate and glycylglycine as acceptor, with the application of Roche Diagnostics/Hitachi 902 Analyzer. The activity of ALP in the liver cytosol was measured by standard method of Hausamen *et al* [28] using 2-amino-2-methyl-1-propanol (AMP) as a buffer, Monoethylamine does not interfere, by using Roche Diagnostics/Hitachi 902 Analyzer.

Protein measurement

The protein content in the liver cytosol was measured according to Lowry method [29].

Access RT-PCR system: protocol

Characteristics of hepatocytes were evaluated by reverse-transcription polymerase chain reaction (RT-PCR). RNA was extracted from the hepatocytes using a TRIZOL reagent RNA purification kit (Invitrogen). cDNA was synthesized using oligo-d (T)15 and Omniscript RT kit.

Statistical analysis

All data were subjected to analysis of mean using one-way variance analysis (ANOVA). Post-hoc comparisons were made using the Benferonni's test. The Statistical Package for Social Sciences (SPSS) for windows version 11.5 was used to analyze the data.

Results

Table 1 shows the effect of BFE on weight gains and the ratio of liver to body weights of rats induced with hepatocarcinogenesis. The results showed that the DEN/AAF control group had the least increase of body weight compared to other groups. The NC and NB groups had a significantly lower ratio of liver weight to body weights compared with the DEN/AAF control group ($p < 0.05$). The DAB groups had a lower ($p<0.05$) ratio compared with the DEN/AAF control group (except DAB_{25}). Treatment with BFE lowered the ALP activity in the liver cytosol of rats that had been induced with DEN and AAF (Table 2). ALP in the DEN/AAF control group was significantly higher than all groups ($p<0.05$). ALP for the DAB_{50} was the lowest, but not significantly different compared to NC and NB_{50} groups. However, the results indicated that BFE could normalize ALP in the DAB Groups. Treatment with BFE also decreased GST activities in the liver cytosol of rats (Table 3). GST in DEN/AAF control group was significantly higher ($p < 0.05$) compared with all groups. GST for the NC group was lower compared with the NB groups significantly, but for NB_{25} was not significant. Furthermore, GST in DAB groups was significantly lower compared with DEN/AAF control group ($p < 0.05$). Like ALP and GST activities, BFE lowered the GGT activities in the liver microsomes of hepatocarcinogenic rats (Table 4). The DEN/AAF control group had significantly higher GGT activities compared with the NC group, while GGT for the NB and DAB groups was significantly lower compared with the DEN/AAF control group ($p <0.05$).

RT-PCR was performed to identify the expression of genes encoding markers in hepatocyte (albumin, α-fetoprotein). DEN/2-AAF model was successfully established to activate the oval cell compartment in rat liver. The hepatic cells expressed hepatocyte-marker albumin. In DEN/AAF control group, they expressed AFP gene. RT-PCR showed expression of albumin gene in all groups. The expression of AFP gene was only detected in DEN/AAF control group, which did not receive BFE (Fig. 1). As shown in Fig.1, the AFP hepatocytes PCR products gave rise to a band at 561bp (sample 4) and 599 bp (sample 6) in gel electrophoresis. As shown in

Table 2: ALP enzyme activity in liver of rats induced with diethylnitrosamine and 2-acetylaminoflourene.

Groups	n	Cytosol ALP (U/I)
Normal control (N)	6	17.61 ± 0.77^{a}
BFE_{25} (NB_{25})	6	$17.31\pm 1.88^{b,a}$
BFE_{50} (NB_{50})	6	$19.19\pm 3.61^{c,a,b}$
BFE_{100} (NB_{100})	6	$22.72\pm 1.24^{d,a,c,f,h}$
DAC control	5	28.46 ± 4.76^{e}
DAB_{25}	5	$23.62\pm 2.06^{f,a,c,d,h}$
DAB_{50}	5	$15.14\pm 1.64^{g,a,c}$
DAB_{100}	5	$20.09\pm 3.67^{h,a,c,b,d,f,g}$

Data are mean ± SD. Values with different letters are significantly different at $p<0.05$. N: normal control group; NB_{25}: normal control treated with BFE_{25}; NB50: normal control treated with BFE_{50}; NB_{100}: normal control treated with BFE_{100}; DAC: DEN/AAF control group; DAB_{25}: DEN/AAF control treated with BFE_{25}; DAB_{50}: DEN/AAF control treated with BFE_{50}; DAB_{100}: DEN/AAF control treated with BFE_{100}; BFE: *Berberis vulgaris* fruit extract.

Table 3: Glutathione S-transferase enzyme activity in liver of rats induced with diethylnitrosamine and 2-acetylaminoflourene.

Group	n	Cytosol GST (µmol/min/mg)
Normal control (NC)	6	$0.112\pm 0.097^{a,b}$
BFE_{25} (NB_{25})	6	$0.308\pm 0.081^{b,d,a}$
BFE_{50} (NB_{50})	6	$0.514\pm 0.141^{c,d}$
BFE_{100} (NB_{100})	6	$0.398\pm 0.151^{d,b,c}$
DEN/AAF control	5	0.885 ± 0.0341^{e}
DEN/AAF+BFE_{25}	5	0.317 ± 0.0908^{f}
DEN/AAF+BFE_{50}	5	$0.576\pm 0.170^{g,c,d}$
DEN/AAF+BFE_{100}	5	$0.522\pm 0.0447^{h,g}$

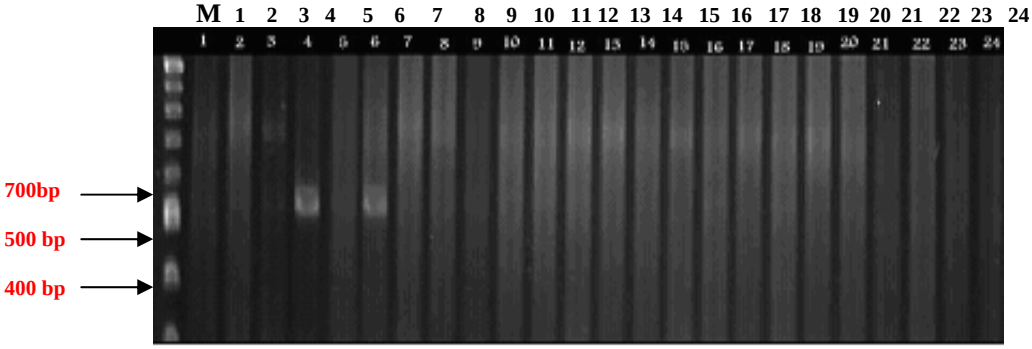
Data are mean ± SD values in µmol/minute/mg of protein. Values with different letters are significantly different at $p<0.05$. N: normal control group; NB_{25}: normal control treated with BFE_{25}; NB50: normal control treated with BFE_{50}; NB_{100}: normal control treated with BFE_{100}; DAC: DEN/AAF control group; DAB_{25}: DEN/AAF control treated with BFE_{25}; DAB_{50}: DEN/AAF control treated with BFE_{50}; DAB_{100}: DEN/AAF control treated with BFE_{100}; BFE: *Berberis vulgaris* fruit extract.

Table 4: Gamma glutamyl transpeptidase enzyme activity in liver of rats induced with diethylnitrosamine and 2-acetylaminoflourene.

Groups	n	GGT Microsome (U/I)
Normal control (NC)	6	0.84± 0.55 ^{a,b,c,d,f,g,h}
BFE_{25} (NB_{25})	6	0.36± 0.29 ^{b,a,c,d,f,h}
BFE_{50} (NB_{50})	6	0.57± 0.48 ^{c,a,b,d,f,h}
BFE_{100} (NB_{100})	6	0.98± 0.90 ^{d,a,b,c,f,g,h}
DEN/AAF control	5	4.55± 1.89^{e}
DAB_{25}	5	1.33±0.81^{f,a,b,c,d,f,g,h}
DAB_{50}	5	2.61± 1.00 ^{g,d,f,h}
DAB_{100}	5	1.18± 0.74 ^{h,a,b,c,d,f,g}

Data are mean ± SD; values in U/I. Values with different letters are significantly different at $p<0.05$. BFE**:***Berberis vulgaris* fruit extract; NC: normal control; NB_{25}, NB_{50}, NB_{100}: normal control treated with BFE_{25},BFE_{50} and BFE_{100}, respectively; DAC: DEN/AAF group; DAB_{25}, DAB_{50}, DAB_{100}**:** DEN/AAF treated with BFE_{25}, BFE_{50} and BFE_{100}, respectively

Figure 1: RT-PCR analysis of AFP gene expression in liver tissue of all groups of rats.



1-3=Normal control group.
4-6=DEN/AAF control group.
7-9=Normal control treated with 25 mg/kg/bodyweight of BFE.
10-12=Normal control treated with 50 mg/kg/bodyweight of BFE.
13-15= Normal control treated with 100 mg/kg/bodyweight of BFE.
16-18=DEN/AAF control treated with 25mg/kg/bodyweight of BFE.
19-21=DEN/AAF control treated with 50 mg/kg/bodyweight of BFE.
22-24=DEN/AAF control treated with 100 mg/kg/bodyweight of BFE.

Fig.2, the albumin hepatocytes PCR products gave rise to a band at 513bp (sample 1) in gel electrophoresis. DNA sequence analysis was done for sample 1, 4, and 6 (reverse and forward). Three samples were sent to RESEARCH BIOLABS SDN.BHD. Co No.341611-M for DNA sequence analysis to confirm RT-PCR analysis. NCBI gene bank confirmed the samples number 4 and 6 are AFP gene (97% forward specific primer and 96% reverse specific primer for sample 4 and 87% forward specific primer and 93% reverse specific primer for sample 6). NCBI gene bank confirmed that the sample number 1 is albumin hepatocyte-specific marker (99% for forward specific primer and 98% for reverse specific primer).

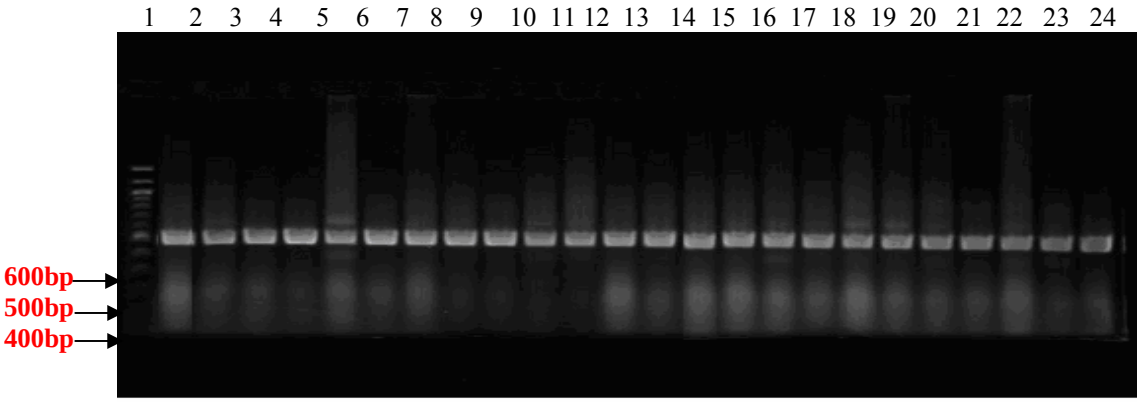
Discussion

Berberis vulgaris fruit is not commonly consumed vegetable in Malaysia. Thus, it is the purpose of this study to determine anticancer properties of *Berberis vulgaris* fruit extract. Few studies have been done on the protective effect of *Berberis vulgaris* fruit against chemical carcinogenesis. Therefore, this study was initiated to investigate the effect of this plant against chemical carcinogenesis. Hepatocarcinogenesis inhibit the regenerating of liver cells following partial hepatectomy, and at higher dosage it may lead to liver cell death. Focal, slowly proliferating cell population, called hyperplastic nodules, occur in the liver before the apprearance of cancer [10]. Proliferation of cells will increase the size of the liver. Therefore, assessment of the liver weights to body weights ratio was used to investigate the change in the liver size. It showed that the rats induced with DEN and AAF had higher liver weight to body weight ratios than that of the normal rats. Peraino *et al* [30] reported a similar result from rats that had been given a diet containing 0.02% AAF. In this study, the ratio of liver weight to body weight of the rats induced with DEN and AAF and given BFE was significantly lower than the rats induced with DEN and AAF without BFE. However, for DAB_{25} this ratio was not significant. When BFE was given to the rats that had been induced with hepatocarcinogens, the ALP activities were reduced. GST activities in liver cytosol of rats induced with DEN and AAF were more than sevenfold higher compared with the normal rats. Kitahara et al [31] and Rahmat et al [32] also reported elevated GST activities in liver cytosol of rats after initiation of hepatocarcinogenesis with DEN and AAF.

GSTs are multifactorial in the detoxification processes. They play an important role in the catalysis of reactions in which glutathione acts as a nucleophile. They serve as an equally important role of binding proteins, as manifested by their storage function for bilirubin in liver [33]. The increased activities of antioxidant enzymes, such as glutathione S-transferase play a major role in repairing the damaged caused to cytoplasmic nuclear and endogenous membranes in liver [34]. GSTs have been reported to reduce the susceptibility of the liver to aminoazo dye-, polycyclic aromatic hydrocarbon-, and aromatic amine-induced carcinogenesis [35]. Therefore, elevated GST activities in rats induced with DEN and AAF are due to the detoxification role of GST. GST activities were reduced after BFE was given to the rats. Probably BFE have reacted with DEN and/or AAF to reduce the cancer-inducing action of the carcinogens. The liver microsomal GGT activities in the DEN and AAF induced rats were higher than normal rats. Fiala *et al* [36] reported that the GSH concentrations and GGT activities were higher in the liver of rats that had been induced with hepatocarcinogenic 3´-methyl-4-dimethylaminoazobenzene. GSH can effectively scavenge reactive oxygen species directly and indirectly through enzymatic reactions. GSH can conjugate with nitric oxide (NO), resulting in the formation of S-nitroso-glutathione adduct, and then be cleaved by the thioredoxin system to release GSH and NO in the form of heme-NO or NO_3^- [37].

GGT is a membrane-bound enzyme that cleaves extracellular glutathione, providing cells with amino acids necessary for intracellular synthesis of new GSH compounds [38]. Kitahara *et al* [31] and Rahmat *et al* [32] reported that the GSH concentration was elevated in DEN and AAF induced rats. Hiramoto *et al* [39] showed that *N*-nitrosodimethylamine and DEN were decomposed, accompanying concomitant release of NO on contact with reactive oxygen species. Therefore, the GSH concentration is elevated because of the need to conjugate with NO from DEN, and this raises the GGT activities. After

Figure 2: RT-PCR analysis of albumin gene expression in hepatocytes of all groups of rats.



1-3=normal control.
4-6=DEN/AAF control.
7-9=normal control treated with 25 mg/kg/bodyweight of BFE.
10-12= normal control treated with 50 mg/kg/bodyweight of BFE.
13-15= normal control treated with 100mg/kg/bodyweight of BFE.
16-18=DEN/AAF control treated with 25 mg/kg/bodyweight of BFE.
19-21=DEN/AAF control treated with 50 mg/kg/bodyweight of BFE.
22-24=DEN/AAF control treated with 100mg/kg/bodyweight of BFE

giving BFE to rats, the GGT activities were reduced. Thus, BFE may have scavenged NO produced from DEN. In other word, BFE could also react with DEN or AAF directly.

The effect of *Berberis vulgaris* fruit extract in DEN/AAF induced hepatocellular carcinoma was studied in female *Sprague dawley* rats. RT-PCR analysis showed expression of AFP only in DEN/AAF control group. RT-PCR as a very sensitive method did not show gene expression of AFP in DEN/AAF groups treated with *Berberis vulgaris* fruit extract. Albumin specific-primer was used as internal control for hepatocytes. It became clear that AFP is a valuable marker in the differential diagnosis of HCC [23].

AFP expression is induced in regenerating liver and liver tumors. DEN is a genotoxic carcinogen that has been shown to induce liver tumors and liver damage [40]. The AFP gene belongs to albumin gene family along with serum albumin, a group-specific component, also known as vitamin D-binding protein, and α-albumin genes. As well as their genes, these proteins are highly homologous in primary structure. All of them are synthesized in liver and secreted into blood serum, providing delivery of their bound ligands to different tissues. All the albumin family genes are located on the same chromosome. The AFP, SA, and a-albumin genes are positioned near each other and have a common direction of transcription. Albumin genes are located on chromosome 5 of mouse, 14 of rat, and on the long arm of chromosome 4 of human (4q11- q13). The expression of these genes is interconnected and has common principles of regulation. At the end of the embryonic period of development, at the same time as the morphological restructuring of the liver, a drastic decrease in AFP blood level and reduction of AFP-producing cell number take place. Simultaneously, SA blood level increases.

This switch is carried out on the transcriptional level. At the same time, it has been shown that both genes can be expressed in the same cell simultaneously. Shortly after birth AFP concentration in blood decreases 10^4-fold. AFP gene expression is repressed reversibly in adult liver. It can be restored during the course of liver regeneration induced by partial hepatectomy, when up to 2/3 of the organ is removed surgically, or by acute CCl_4 intoxication that causes necrosis of the hepatocytes bordering central veins. Simultaneously with AFP induction, SA synthesis decreases. The most significant effect is observed in mice [41].

In this study, AFP gene expression in DEN/AAF control group might be due to DEN/AAF intoxication that caused necrosis of the hepatocytes. Hepatocyte localization within the liver plate or outside it is the defining factor that regulates the activity of AFP synthesis on a cellular level [41]. Regulation of the AFP gene expression has been shown by a number of investigators to be extremely complex. Its expression is limited to a small number of tissues during a specific time in development [40]. It has been generally accepted that the liver contains cells with stem-like properties and that these cells can be activated to proliferate and differentiate into mature hepatocytes and cholangiocytes under certain pathophysiologic circumstances [42, 43]. These cells might be related to the so-called "oval cells", originally identified by Farber as immature epithelial cells with oval shaped nuclei and scant cytoplasm [44]. There are many common features between immature embryonic hepatic cells and oval cells such as expression of AFP. It is reasonable to assume that oval cells may be a direct progeny of resident undifferentiated liver stem cells. A population of hepatic stem cells that exist in developing mouse liver and these cells may represent the resident hepatic stem cells which possess multilineage differentiation potential and self-renewing capability [45, 46]. Chen *et al* [47] reported that transfused oval cells through caudal vein can migrate into the parenchyma of the liver and are settled there. Possible explanations for the reinitiation of AFP synthesis by neoplastic hepatocytes include either increased transcription of AFP gene or post-translational modification affecting AFP production. In rats which have been exposed to chemical carcinogens or with HCC, AFP production is roughly proportional to the amount of transplantable mRNA present. Steady state AFP transcription may begin prior to the development of histologically or symptomatic hepatocellular carcinoma [48].

The reproduction of AFP in adults is associated with hepatocellular carcinoma and germ cell tumors such as yolk sac tumor. Although the precise mechanism of this regulation is not fully understood, the expression of AFP is regulated at the transcriptional level with networks of interaction among the promoter, enhancers, and silencers of the AFP gene. Sets of nuclear factors that are abundantly expressed in hepatocytes are required in this regulation. These molecules are collectively called liver-enriched transcription factors, which include the CCAAT/enhancer-binding protein (C/EBP) family and the hepatocyte nuclear factors (HNF) family. The liver-enriched transcription factors are expressed in embryonic endoderm, and they are assumed to be important in the development of visceral yolk sac and the gene expression of plasma proteins in yolk sac cells. High expression of HNF-1 at early gestation is observed in the yolk sac, which is associated with the synthesis of plasma proteins. Mouse embryonal stem cells express HNF-3β and HNF-4 during development of the embryoid body with visceral yolk sac structures, and the expression of these transcription factors starts before AFP and transthyretin expression [49]. In the present study, AFP as a tumour marker showed successful induction of hepatocarcinogenesis in DEN/AAF group. In conclusion, RT-PCR analysis showed AFP gene expression in DEN/AAF groups treated with *Berberis vulgaris* has been blocked, as a sign of anticancer property of *Berberis vulgaris* fruit.

Conclusion

This study contributes to the new finding towards anticancer agent from a plant fruit, *Berberis vulgaris* contributing to the effort of treatment and suppressive of the cancer diseases. Our findings suggest chemopreventive agent against hepatocarcinogenesis by *Berberis vulgaris* fruit arise from experimental studies in animal model. These results indicated that BFE, containing many beneficial nutrient compositions, may function to suppress the severity of cellular and molecular

changes of DEN-initiated and AAF-promoted liver tumours significantly.

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Table 1. Body weight gain, liver weight to body weight ratio and liver enzyme activities in eight groups

Group	Number	Weight gain	Liver /body weight (g/100g)	Cytosol ALP (U/I)	Cytosol GST (µmol/min/mg)	GGT Microsome (U/I)
Normal control (NC)	6	109 ± 11.14	0.027± 0.002^{a,b,c,d}	17.61± 0.77^{a}	0.112± 0.097 ^{a,b}	0.84± 0.55 ^{a,b,c,d,f,g,h}
BFE_{25} (NB_{25})	6	133 ± 11.44	0.028± 0.001^{b}	17.31± 1.88^{b,a}	0.308± 0.081 ^{b,d,a}	0.36± 0.29 ^{b,a,c,d,f,h}
BFE_{50} (NB_{50})	6	36 ± 11.86	0.026± 0.004^{c}	19.19± 3.61^{c,a,b}	0.514± 0.141 ^{c,d}	0.57± 0.48 ^{c,a,b,d,f,h}
BFE_{100} (NB_{100})	6	69 ± 14.22	0.025± 0.001^{d}	22.72±1.24^{d,a,c,f,h}	0.398± 0.151 ^{d,b,c}	0.98± 0.90 ^{d,a,b,c,f,g,h}
DEN/AAF control	5	16 ± 20.94	0.031± 0.003^{e}	28.46± 4.76^{e}	0.885± 0.0341^{e}	4.55± 1.89^{e}
DEN/AAF+BFE_{25}	5	50 ± 25.02	0.03 ± 0.003^{f,e}	23.62± 2.06^{f,a,c,d,h}	0.317± 0.0908 ^{f}	1.33±0.81^{f,a,b,c,d,f,g,h}
DEN/AAF+BFE_{50}	5	83 ± 18.07	0.027± 0.001^{g}	15.14± 1.64^{g,a,c}	0.576± 0.170 ^{g,c,d}	2.61± 1.00 ^{g,d,f,h}
DEN/AAF+BFE_{100}	5	51 ± 11.47	0.023± 0.003^{h}	20.09± 3.67^{h,a,c,b,d,f,g}	0.522± 0.0447 ^{h,g}	1.18± 0.74 ^{h,a,b,c,d,f,g}

Data are mean ± SD; values in U/I. Values with different letters are significantly different at p<0.05. BFE**:***Berberis vulgaris* fruit extract; NC: normal control; NB_{25}, NB_{50}, NB_{100}: normal control treated with BFE_{25},BFE_{50} and BFE_{100}, respectively; DAC: DEN/AAF group; DAB_{25}, DAB_{50}, DAB_{100}**:** DEN/AAF treated with BFE_{25}, BFE_{50} and BFE_{100}, respectively

a significant p<0.05 compare NC, b significant with NB 25, c significant p<0.05 compare NB50,

d significant p<0.05 compare NB100, e significant p<0.05 compare DAC control, f significant p<0.05 compare DAB 25, g significant p<0.05 compare DAB 50, h significant p<0.05 compare DAB 100