

Therapeutic Effects of Korean Red Ginseng Extract in Egyptian Patients with Chronic Liver Diseases

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Hepatocellular carcinoma (HCC) is the fifth most common malignancy in the world and complicates liver cirrhosis related to hepatitis C virus (HCV) in many cases. We evaluated the therapeutic effect of Korean red ginseng extract (KGE) in patients with chronic liver diseases. Thirty male and female patients with HCC and another thirty with liver cirrhosis were included. Each category was divided into two groups; the first was used as control group, and received medical therapy only and the second group received the medical therapy supplemented with KGE capsules. The treated group with HCC received three KGE capsules/day (900 mg) while the treated group with HCV received two KGE capsules/day (600 mg) for 11 weeks along with their medical therapy. All patients were subjected to clinical examination and laboratory investigations, including liver function tests (at baseline, after 6 weeks of treatment and at the end of the study) and abdominal ultrasonography. Patients showing focal hepatic lesions were subjected to triphasic spiral abdominal computerized tomography and alpha-fetoprotein (AFP). HCV RNA was determined quantitatively by Roche for patients in the HCV group. Results showed that the medical therapy alone failed to normalize the liver enzymes or decrease the virus concentration. KGE administration induced a significant improvement in liver function tests, decreased the tumor marker (AFP) levels, and decreased the viral titers in HCV patients. Thus, KGE demonstrated powerful therapeutic effects against HCV and liver cancer.

Keywords: *Panax ginseng*, Neoplasms, Egypt, Hepacivirus, Korean ginseng, Liver diseases

INTRODUCTION

Hepatocellular carcinoma is one of the world's deadliest cancers, ranking third among all cancer-related mortality. Most cases occur in Asia and sub-Saharan Africa, where viral hepatitis is endemic. The incidence is rising in the West, likely due to the increase in patients infected with hepatitis C during the latter half of the last century [1]. This incidence is increasing worldwide, between 3% and 9% annually [2]. In Egypt, the annual prevalence of hepatocellular carcinoma (HCC) has increased significantly during the past decade. HCC has been reported to account

for about 4.7% of the chronic liver disease patients, where the epidemiology of HCC is characterized by marked demographic and geographic variations [3]. The liver, unique in its capacity for regeneration following injury, also gives rise to this malignancy, commonly associated with an inflammatory state of advanced fibrosis or cirrhosis. Potentially curative therapies can be offered to approximately 30% of patients, but these are complicated by a high rate of recurrence [4].

Encouraging progress has been made in understanding

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Received 18 Oct. 2010, Revised 7 Feb. 2011, Accepted 7 Feb. 2011

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the molecular pathogenesis of cancer [1,4]. Discoveries of the signal transduction pathways, cascades of protein-protein interactions transmitting information from the cell surface to the nucleus, and of their link to tumour biology, are particularly impressive. Several key mouse models have been instrumental in defining the pathogenesis of HCC by introducing genetic alterations into one or more aetiological pathways that can be targeted exclusively to the liver. Moreover, these programmed manipulations can be introduced systematically, not only in this specific organ, but also at defined times during development, growth, and aging of the liver.

For more than 3,000 years, ginseng has been used in Asia for the treatment of various medical disorders. Since the 18th century, it has been one of the most commonly used herbal medicines in Europe and North America [5]. According to a survey study, ginseng is the second most popular herbal medicine in the United States [6]. Several types of ginseng have been identified to date, including those of the *Panax* genus. The best characterized species in this genus are *Panax ginseng* C. A. Meyer [5]. Recently, 20-O-(β -D-glucopyranosyl)-20(S)-protopanaxadiol (IH-901), a novel ginseng saponin metabolite, formed from ginsenosides Rb1, Rb2, and Rc was isolated and purified after giving ginseng extract orally to humans and rats [7]. IH-901 has been shown to enhance the efficacy of anti-cancer drugs in cancer cell lines previously resistant to several anticancer drugs [8,9], to exhibit antigenotoxic and anticlastogenic activity in rats concurrently treated with benzo(a)pyrene [10], and to induce apoptosis [11,12]. These studies found that the antitumor activity of IH-901 is attributable to the induction of apoptosis. The aim of the current study was to evaluate the therapeutic effects of Korean red ginseng extract in Egyptian patients with chronic liver diseases.

Patients and methods

After receiving approval from the Ethical Committee of the National Research Center, Egypt, patients were recruited. They signed informed consent forms at the Hepatology Outpatient Clinic of the Internal Medicine Department, National Liver Institute, Monoufia University. The study was conducted from May till August 2008.

A full history was taken including determining whether there was a past history of schistosomiasis, as was a thorough clinical examination, laboratory investigations (liver function tests: aspartate transaminase (AST) and alanine transaminase (ALT), serum bilirubin (total and direct), serum total proteins, serum albumin, prothrombin concentration), and abdominal ultrasonography. Patients with

focal hepatic lesions were subjected to triphasic spiral abdominal computed tomography (CT) and alpha-fetoprotein (if not previously done). Hepatitis C virus (HCV) RNA was determined quantitatively by Roche for patients in the HCV group. All biochemical analyses were carried out at Food Toxicology & Contaminants Department, National Research Center. Then, patients were enrolled, according to the following inclusion/exclusion criteria.

Inclusion criteria

Patients (1.) must be between 40 and 80 years old, (2.) of either gender, (3.) be diagnosed with cirrhotic liver disease, and (4.) have evidence of a malignant focal hepatic lesion.

Exclusion criteria

Patients were excluded for the following reasons: (1.) they were diagnosed with a systemic disease, such as a renal, cardiac, or respiratory disease, (2.) patients had malignancies that originated extrahepatically or (3.) if they displayed hepatic encephalopathy at time of the study.

Finally, we were left with thirty patients who had HCC and a mean age of 52.3 ± 8.1 and thirty patients who had HCV with a mean age of 47.8 ± 18.2 . The HCC patients within each category were divided into two groups (15 patients/group), and the first group served as a control (11 males, 4 females). The control group received only their medication with no ginseng administration, while the second group served as the treated group (11 males, 4 females) and received their medical treatment, supplemented with three ginseng capsules (900 mg/day). The HCV patients were also divided into two groups (15 patients/group); the first group served as a control (8 males, 7 females) and received their medical treatments and no ginseng. The second group served as the treated group and received their medical treatments supplemented with two ginseng capsules per day (600 mg/day). All patients continued the study for 11 weeks. During this period, all laboratory investigations were conducted at baseline, at the sixth week (half way) and at the eleventh week (end of study period). These laboratory investigations included a biochemical analysis and the other tests suggested by the physicians. Two females in the second group of HCC patients stopped taking ginseng on day 51 of the study. Thus, we excluded these two females from the control group, and the study continued with the remaining thirteen patients (11 males, 2 females). On the other hand, six patients in the HCV group stopped taking ginseng on day 25 and did not complete the study. Consequently, we discarded these six patients from the control group and

the study continued with the other patients (4 males and 5 females). All laboratory investigations were carried out at the Internal Medicine Department labs. These labs operate under a quality assurance system according to ISO/IEC 17025.

Statistical analyses

The compiled data were analyzed using EPI Info ver. 6.2 (CDC, Atlanta, GA, USA) and the SPSS ver. 7.5 (SPSS Inc., Chicago, IL, USA). ANOVA and the chi-squared test were used to study the pattern of distribution of different variables. Multiple correlation coefficients $\{r\}$ were used to determine the correlation of the studied parameters to each other. A p -value of less than 0.05 was considered to indicate statistical significance. All data are presented as means \pm SE.

RESULTS

The present controlled study involved 26 patients suffering from HCC and 18 patients suffering from liver cirrhosis. Demographic data including age, gender, smoking, and residence between the HCC group and cirrhotics is presented in Table 1. Results indicated that the mean age of HCC patients (52.3 \pm 12.1) was significantly higher than that of cirrhotics (47.8 \pm 18.2). The distribution of gender among the HCC and cirrhotic patients showed that percentage of males (42.3% and 44.4%) was lower than that of females (57.7% and 55.6%), respectively. However, the number of non-smoking cirrhotic patients (72.2%) was higher than the HCC patients (61.5%).

Table 1. Distribution of age, gender, residence, and smoking between the hepatocellular carcinoma (HCC) group and cirrhotic patients

	HCC patients (n=26)	Cirrhotics (n=18)
Age	52.3 \pm 12.1	47.8 \pm 18.2
40-50	10 (38.5)	8 (44.4)
>50	16 (61.5)	10 (55.6)
Gender		
Male	11 (42.3)	8 (44.4)
Female	15 (57.7)	10 (55.6)
Smoking		
Yes	6 (23.1)	3 (16.7)
Ex	4 (15.4)	2 (11.1)
n	16 (61.5)	13 (72.2)
Residence		
Rural	14 (53.9)	7 (38.9)
Urban	12 (46.2)	11 (61.1)

Values are presented as mean \pm SE or number (%).

Regarding the area of residence, the number of HCC patients was higher in rural areas (53.9%) compared with cirrhotic patients (38.9%). However, the number of urban patients was higher in those with cirrhosis (61.1%) than those with HCC (46.2%).

Radiological (ultrasound and spiral CT) data concerning the focal hepatic lesions are presented in Table 2. Results revealed that the focal lesions in the right lobe were significantly more likely than either bilateral multiple focal lesions and focal lesions in left lobe. The percentage of patients having HCC in addition to liver cirrhosis (65.4%) was significantly higher than that of those without liver cirrhosis (34.6%). Furthermore, the absence of portal vein thrombosis was significantly higher.

The classification of patients according to Child-Pugh scoring system is presented in Table 3. The results indicated that the percentage of grade C among HCC patients (57.7%) was the highest, followed by grade B (30.8%) and grade A (11.5%). However, the only significant difference was detected between percentages of patients in grades B and A, and between those patients in grades C and A. Among the group with cirrhosis, there was no significant difference regarding Child-Pugh classification between class A and B, although there was a significant difference between class C and the other two classes.

The biochemical analysis for male and female patients presented in Table 4 revealed that both of the control groups (males and females) and the Korean red ginseng extract (KGE)-treated group (with HCC or cirrhotic) showed a significant increase in ALT at the beginning of the study. Moreover, the increase in ALT activity was more pronounced in females. All patients showed a significant decrease in ALT activity after the administration of KGE capsules at 6 and 11 weeks. Results also indicated that the

Table 2. Results of ultrasonographic data for the hepatocellular carcinoma (HCC) group

	HCC patients	
	n=26	%
Fatty liver	14	53.8
Right lobe	7	26.9
Left lobe	5	19.2
Both lobes	--	--
Cirrhosis		
Yes	17	65.4
No	9	34.6
Portal vein thrombosis		
Yes	8	30.7
No	18	69.3

Table 3. Classification of patients according to the Child-Pugh scoring system

Child-Pugh score	HCC patients		Cirrhotics	
	n=26	%	n=18	%
A	3	11.5	3	16.7
B	8	30.8	5	27.7
C	15	57.7	10	55.6

HCC, hepatocellular carcinoma.

activity of ALT remained increased in the control group and this increase was pronounced at week 11 although these patients received their medication. A significant decrease was found in the group that received their medication plus KGE capsules (900 mg/day for HCC patients and 600 mg/day for cirrhotics), although the level of the liver enzymes was still higher than the reference range.

The current results also indicated that both groups of patients showed a significant increase in AST activity at the beginning of the study. The activity of this enzyme was higher in males than females and decreased significantly after the administration of KGE. This decrease was 13.4% at week 6 and 23.4% after 11 weeks of KGE administration. On the other hand, the activity of AST increased continuously in the control group and reached a maximum by week 11. However, AST levels decreased continuously in the KGE-treated group and reached their minimum by week 11.

The data presented in Table 4 revealed that serum

total protein (TP) in the two groups of patients showed an insignificant decrease compared with the reference range (6.4-8.3 g/dL). At the beginning of the study, both groups had similar concentrations of serum TP, although it was higher in the group subjected to KGE treatment than the control group. On the sixth week of KGE treatment, TP was increased and reached 6.98 g/dL and 7.24 g/dL in males and females, respectively. A further increase in serum TP was recorded by the eleventh week of KGE treatment and was 7.44 g/dL and 7.66 g/dL in males and females, respectively. Serum TP also increased in the control patients during the study period, likely indicating an effect of the medication. However, the combination of KGE treatment plus the medication resulted in a pronounced improvement in the serum TP, which reached the reference range level in both males and females patients with HCC or cirrhosis.

The effects of KGE administration on serum albumin level in the male and female patients (Table 4) revealed that serum albumin level in both groups was lower than the reference range (3.3-5.0 g/dL). Moreover, the serum albumin level was higher in females than in males of the same group. Administration of KGE capsules (900 mg/day for HCC patients and 600 mg/day for cirrhotics) elevated the serum albumin level after 6 weeks of administration. However, the administration of KGE at the same dose for both studied groups resulted in a significant improvement in serum albumin level that reached the normal reference range. This improvement was more pronounced in males

Table 4. Effect of ginseng administration on liver function in male and female patients with chronic liver disease during the study period (mean±SE)

Parameters	Gender	Baseline		6th week		11th week	
		Control	KGE	Control	KGE	Control	KGE
ALT (IU/L)	M	69.3±10.4 ^a	59.2±8.4 ^d	69.6±7.5 ^a	50.8±7.2 ^b	82.9±8.9 ^b	36.1±3.9 ^c
	F	77.1±14.8 ^a	103.6±15.7 ^a	94.7±15.4 ^b	85.6±10.5 ^b	165.4±46.5 ^c	55.0±6.9 ^c
AST (IU/L)	M	64.0±11.4 ^a	72.8±9.9 ^a	71.9±8.5 ^b	63.1±8.7 ^b	91.3±8.7 ^c	55.8±8.0 ^c
	F	67.3±17.1 ^a	109.6±19.1 ^a	78.6±14.2 ^b	93.6±15.1 ^b	150.9±26.7 ^c	74.7±11.2 ^c
TP (g/dL)	M	5.6±0.1 ^a	6.5±0.1 ^a	5.9±0.1 ^a	7.0±0.1 ^a	6.2±0.1 ^b	7.4±0.1 ^c
	F	5.6±0.2 ^a	6.7±0.1 ^a	6.0±0.2 ^a	7.2±0.2 ^b	6.4±0.2 ^b	7.7±0.2 ^b
Albumin (g/dL)	M	3.2±0.1 ^a	3.5±0.1 ^a	3.1±0.1 ^a	3.5±0.1 ^a	3.1±0.2 ^a	4.0±0.1 ^b
	F	3.0±0.3 ^a	3.4±0.2 ^a	3.2±0.2 ^b	3.6±0.2 ^a	3.3±0.2 ^c	3.9±0.2 ^b
TB (mg/dL)	M	1.7±0.2 ^a	1.5±0.2 ^a	2.0±0.3 ^b	1.3±0.2 ^b	2.1±0.3 ^c	1.0±0.1 ^c
	F	0.9±0.2 ^a	1.5±0.4 ^a	1.3±0.3 ^b	1.3±0.3 ^b	1.7±0.4 ^c	1.1±0.3 ^c
DB (mg/dL)	M	0.9±0.1 ^a	0.8±0.1 ^a	1.2±0.3 ^b	0.7±0.1 ^a	0.9±0.2 ^a	0.5±0.1 ^b
	F	0.3±0.1 ^a	0.7±0.3 ^a	0.7±0.3 ^b	0.7±0.4 ^a	0.9±0.4 ^c	0.4±0.2 ^b
Prothrombin concentration (%)	M	66.9±4.1 ^a	75.5±3.0 ^a	67.0±3.4 ^a	81.9±2.8 ^a	69.5±3.1 ^a	86.0±3.7 ^b
	F	64.0±8.1 ^a	73.2±4.6 ^a	71.1±4.8 ^b	79.7±4.7 ^a	70.7±6.3 ^b	87.2±4.2 ^b

Within each row, different superscript letters indicate a significant difference ($p \leq 0.05$).

KGE, Korean red ginseng extract; ALT, alanine transaminase; AST, aspartate transaminase; TP, total protein; TB, total bilirubin; DB, direct bilirubin.

than in females. Albumin levels in control patients did not change significantly during the study period. However, it started a significant increase after 6 weeks of KGE administration.

The total bilirubin (TB) level was significantly higher in male than female patients in the two groups. The concentration of TB at the starting date was higher than the normal reference range (0.2-1.0 mg/dL). The TB concentration was higher in males than in females in the control group, while it was higher in females than in males in the group with KGE administration. Administration of KGE capsules at the two doses tested for 6 weeks resulted in a significant decrease in TB in both male and female patients. More improvement was also achieved when the treatment period was prolonged to 11 weeks, and the benefits were more pronounced in males than in females at week 11.

Results indicated that direct bilirubin (DB) concentration was also affected in a similar manner to the TB concentration in all patients at the start of the study. The data related to DB revealed that DB concentration was significantly higher than the normal reference range (up to 0.3 mg/dL) in both groups of patients at the beginning of the study. The same data showed that DB was higher in males than females. Administration of KGE capsules at a dose of 900 mg/day for the HCC patients and 600 mg/day for the cirrhotics for 6 weeks was associated with a significant improvement in DB concentration, although it was still higher than normal. Moreover, the administration of KGE at the same dose for 11 weeks resulted in a more significant decrease in DB, although it did not normalize. Furthermore, the results also indicated that the effects of KGE were more pronounced in female patients than in males. The concentration of DB in the control patients group continued to increase from time zero until the 6th week, and then it started to decrease, presumably due to the medication they received. However, the concentration of DB in the KGE-treated group decreased during the first 6 weeks and continued to decrease through the 11 week, although the treatment with KGE for this period did not normalize DB.

The prothrombin concentration (PC) for males and females in the control patients who received their prescribed treatments only was also lower in males than in females. Administration of KGE plus the medication resulted in a significant increase in PC by week 6, and this improvement was more pronounced in males than females. In a similar manner, the combined treatment with KGE capsules and the medication for 11 weeks resulted in a significant improvement in PC back towards the normal reference range. Moreover, there was no significant

difference between male and female patients at the end of week 11. No significant improvement was found in the control patients subjected to medical treatment only during the study period. PC increased gradually from the starting date until week 6, but did not change significantly after that. The KGE-treated group of patients showed a gradual increase in PC, starting on the first day of KGE administration and continuing until the end of week 11.

At the end of the study period, patients with HCV and HCC (males and females) were separated, and the concentrations of HCV and alpha-fetoprotein (AFP) were evaluated. The results presented in Fig. 1 indicate that administration of KGE capsules at a dose of 600 mg/day for both male and female patients with HCV resulted in a significant decrease in the viral concentration compared with the control patients with HCV. Moreover, the reduction in viral concentration was more pronounced in males than females.

The results presented in Fig. 2 also indicated that AFP was significantly higher in the control patients. Moreover, the level of AFP was higher in males than females. Administration of KGE at a dose of 900 mg/day for 11 weeks resulted

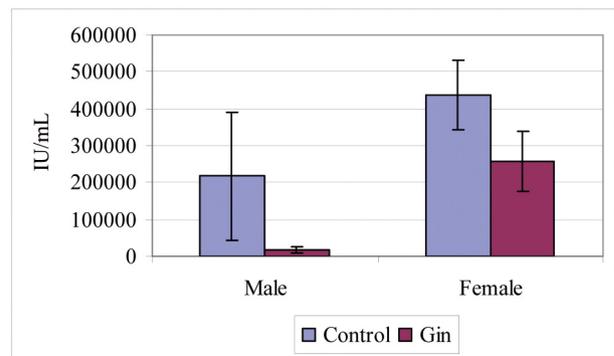


Fig. 1. Effects of ginseng administration on viral concentration in male and female patients with cirrhosis who were treated with ginseng capsules (600 mg/day) for 11 weeks.

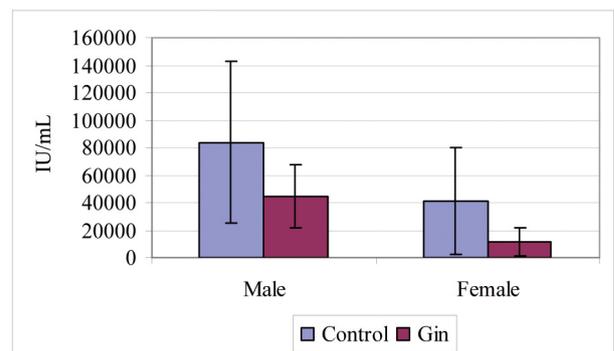


Fig. 2. Effects of ginseng administration on alpha-fetoprotein in male and female patients with hepatocellular carcinoma and treated with ginseng capsules (900 mg/day) for 11 weeks.

in a significant decrease in AFP, and this decrease was more pronounced in females than in males.

DISCUSSION

HCV infection is a major cause of chronic hepatitis affecting approximately 175 million people worldwide [13]. The prevalence of cirrhosis from chronic HCV infection and the incidence of its complications are expected to increase all around the world over the next 10-20 years [13,14]. Currently, intravenous drug use, unprotected sex with multiple partners, and viral exposure during medical procedures, such as surgery, dialysis, and dental treatment, are factors associated with the highest degree of risk for HCV infection [15,16]. Healthcare employees are at risk for acute hepatitis C through accidental exposure, such as needle stick injury; however, recent reports indicate that the risk for HCV transmission after needle stick injury is lower than previously believed (mean value 0.75%; in Europe 0.42%; in Eastern Asia 1.5%) [17]. Risk factor patterns apparently vary according to geography. For example, within many Western countries, intravenous drug use is the greatest risk factor, with sexual transmission and medical practices representing other less common risk factors [18]. Conversely, in Egypt, occupational exposure seems to be the greatest hazard, with intravenous drug use and sexual transmission being less important [19,20]. Although guidelines exist for the management of chronic hepatitis C [13], they do not specifically address acute hepatitis C. After exposure to HCV, there is a window of 1-3 weeks before serum HCV RNA can be detected. In patients in whom symptoms are developing, the incubation period between exposure and appearance of symptoms can range from 2 to 12 weeks [21]. The most common symptoms are fatigue and jaundice, with dyspepsia and abdominal pain often being reported [22]. Given that most symptoms are non-specific, many patients do not consult a physician and do not receive a diagnosis during the acute phase [23]. On the other hand, HCC is the fifth most common cancer in the world. Approximately 315,000 cases of HCC are diagnosed each year, accounting for 4.1% of all new human cancer cases [24]. The highest incidence rates of HCC are in Asia and sub-Saharan Africa, where there is a marked increase in incidence in younger age groups [25]. Nevertheless, both incidence and mortality rates are also increasing in some countries in North America and Europe [26,27]. Although various non-surgical treatment modalities have been developed and surgical techniques are much improved, none of these

therapies has significantly improved the extremely poor prognosis of patients with HCC. Thus, searching for new compounds for the treatment of HCC is the aim of numerous studies, and many works have focused on plant-derived compounds that are known to have curative potential from traditional medicines.

In the current study, we evaluated the anticancer and protective effect of KGE in Egyptian patients with liver cirrhosis and/or liver cancer. Male and female patients were administered medical treatments supplemented with 900 mg/day KGE for liver cancer patients and 600 mg/day KGE for patients with liver cirrhosis for 11 weeks. The doses of KGE used in the current study were based on our previous work in experimental animals [28] and others. The results revealed that both types of patients (HCC and cirrhotics) showed a significant increase in all biochemical parameters tested. In males and females with cirrhosis of the liver, liver enzymes showed a significant increase, beginning on the starting day and did not normalize or even decrease significantly, although they received medical therapy alone. The first indication of hepatic injury is an elevated ALT level, which can occur 4-12 weeks after viral exposure [29]. Other criteria that can aid in diagnosing HCV infection include significantly elevated ALT levels ($>10\times$ upper limit of normal [ULN] or $>20\times$ ULN), known or suspected exposure to HCV, and increasing numbers of reactive proteins in a recombinant immunoblot assay confirmation test [30,31]. Male and female patients in both groups showed an increase in ALT, AST, and urea, accompanied by significant decreases in total protein and albumin. The increases in transaminases in HCC and HVC patients are indicative of changes in the hepatic tissues and biliary system [28,32], whereas increased levels of urea with the decreased levels of total protein and albumin may indicate protein catabolism as a result of liver damage and/or renal dysfunction [33,34]. Moreover, HCV infection causes a liver disease that becomes chronic in 70% to 80% of patients and leads to severe complications, such as cirrhosis and liver cancer after many years [35]. The current results also indicate that the viral concentration was very high in male and female patients with an HCV infection. Moreover, female patients had higher viral concentrations than males. AFP was found to be very high in male and female patients with HCC. Generally, the current results indicate that medical therapy alone was not fully effective in either male or female patients with HCC or HCV.

Both male and female patients who received their medical therapy supplemented with KGE showed a

significant improvement in all the parameters tested. Liver enzymes showed a significant decrease in both groups of patients. Moreover, the viral concentration decreased significantly in HCV patients. The response to this protocol was higher in males than in females. A 91.8% decrease in viral concentration was found in males who received the antiviral therapy plus two KGE capsules per day for 11 weeks, whereas a 41.6% decrease was found in female patients who received the same dose for the same period. On the other hand, AFP decreased significantly in males and females who took their medical therapy along with three KGE capsules per day for 11 weeks. Furthermore, the improvement in AFP was more pronounced in females than males; the reduction ratio reached 71% in females and 47% in males.

According to Attele *et al.* [36], the major components of *Panax ginseng* cultivated in Korea are ginsenosides. Numerous researchers have contributed to the accumulation of evidence that ginsenosides are responsible for many biological activities including anti-inflammatory, anti-allergic, and anti-tumour activities seen in cell culture or *in vivo* studies following intraperitoneal or intravenous injection of experimental animals [37,38]. However, others have proposed the concept that these plant glycosides act as prodrugs that are metabolized to active form by deglycosylation by intestinal bacterial [39,40]. Many studies have revealed that the anticancer activities of ginsenosides after oral administration are probably attributable to metabolites formed by intestinal bacteria [41,42] and fatty acid esterification [43]. The components of red ginseng are known as 20(*S*)-ginsenoside Rg3, ginsenosides Rh2, Rs1, or Rs2, Rs3, Rs4 and Rg5, plus notoginsenoside-R4 in the protopanaxadiol group, and 20(*R*)-ginsenoside Rg2, 20(*R*)-ginsenoside-Rh1, ginsenosides Rh4 and F4 in the protopanaxatriol group. Malonyl-ginsenoside-Rb1, Rb2, Rc, and Rd are found only in white ginseng [44]. However, Bae *et al.* [45,46] isolated and identified a novel ginseng saponin metabolite, formed from ginsenosides Rb1, Rb2, and Rc by the human intestinal bacteria deglycosylation, after giving ginseng extract to humans and rats. This novel ginseng saponin was 20-O-(β -D-glucopyranosyl)-20(*S*)-protopanaxadiol (IH-901, compound K, or M1). Because IH-901 was detected as one of the major metabolites in urine and blood after the oral administration, IH-901 is likely a major form of protopanaxadiol saponin absorbed from the intestine [47,48]. IH-901 inhibits glucose uptake by tumour cells [49], and exhibits an anti-metastatic effect *in vivo* [50]. Moreover, IH-901 possesses chemopreventive and chemotherapeutic potential, because it shows antigenotoxic

and anticlastogenic activity induced by benzo[a]pyrene [10], anti-tumour activity in cisplatin-resistant pulmonary adenocarcinoma cells [9], and reversal of multidrug resistance in tumor cells [8]. However, the anti-proliferation and apoptotic actions of IH-901 in human hepatocellular carcinoma cells are not clearly understood.

Present cancer chemotherapeutics exert part of their pharmacological effects by triggering apoptotic cell death, and the induction of apoptosis in tumor cells has become a strategy in cancer treatment [51]. Apoptosis can be initiated by extracellular and intracellular signals that trigger a complex machinery of pro-apoptotic proteases and mitochondrial changes. It is the integration of multiple survival and death signals that determine whether a cell survives or undergoes apoptosis. Moreover, IH-901 was shown to induce apoptosis in several different cancer cell lines [12,52], and several apoptosis-related molecules play important regulatory roles in IH-901-induced tumor cell death [53,54]. Along the same lines, Ming *et al.* [55] suggested that IH-901 induced apoptotic cell death concurrently with cell cycle arrest in SMMC7721 cells. Similar effects of IH-901 in inhibiting the cell growth have been reported previously in other cancer cell lines [54].

Cytochrome *c* normally resides in the mitochondrial intermembrane space, where it serves as a transducer of electrons in the respiratory chain. However, in chemical-induced apoptosis, mitochondria also play a central role in the commitment of cells to apoptosis [56]. It has been demonstrated that several anti-tumor drugs with diverse intracellular targets can cause mitochondrial release and cytosolic accumulation of cytochrome *c* [57]. After release from mitochondria, cytochrome *c* binds with Apaf-1 and participates in the activation of caspase-9 (an initiator). Activated caspase-9 then activates caspase-3 (an effector). Initiator caspases and effector caspases act together to augment the death signal and finally lead to a unilateral process of apoptosis [58,59]. Previous studies have established that IH-901 plays an important role in triggering the mitochondria-mediated apoptosis pathway in different cancer cell lines, in which caspases are the central components [11,60]. Ming *et al.* [55] showed that cytochrome *c* was upregulated, and procaspase-3 and procaspase-9 were downregulated in a concentration-dependent manner by IH-901 treatment and thus activated the proteolysis. Moreover, cytochrome *c* can initiate a complex series of caspase activation events, ultimately resulting in apoptosis. The interaction between the anti-apoptotic and pro-apoptotic Bcl-2 family members has been shown to regulate this event [61]. It is commonly believed that Bcl-2 and its homolog Bcl-XL have a role

in preventing mitochondrial membrane disruption and the release of cytochrome *c* and other pro-apoptotic factors, while Bax/Bak-like proteins promote these events [62,63]. The total expression rate of these two contrary proteins might partly indicate the fate of cells.

On the other hand, IH-901-induced apoptosis in SMMC7721 cell lines might act through increased expression of p53, a tumor suppressor gene [55]. Activation of p53 changes the ratio of Bcl-2/Bax and Bcl-XL/Bax, leading to the release of cytochrome *c* from mitochondria. Thus, proteins from the Bcl-2 family might act as downstream signal carriers in the process of IH-901-induced apoptosis in the human hepatocellular carcinoma cell line SMMC7721.

Sterols, other components of *Panax ginseng*, also have antineoplastic activity. Studies have shown that sterol-enriched (such stigmasterol and sitosterol) diets reduce the plasma cholesterol concentrations [64,65], have anti-inflammatory actions [66,67], and display analgesic and anti-mutagenic effects [67,68]. Moreover, Yun *et al.* [69] used a mixture of Rg3 and Rg5, present in large amounts in red ginseng, and showed that they reduced the incidence of lung adenoma induced by benzo(a) pyrene in mice to 45.0% (inhibition rate, 25.0%). They concluded that Rg3 + Rg5 had anticarcinogenic effects. Similar to the current observations, Nishino *et al.* [70] reported that oral administration of red ginseng extracts significantly suppressed spontaneous liver tumour formation in C3H/He male mice.

According to Antonelli *et al.* [71], HCV is able to affect B-lymphocytes through the CD receptor, inducing a polyclonal B-cell activation that leads to the production of cryoglobulins, rheumatoid factor, and several autoantibodies. On the other hand, epidemiological evidence clearly indicates that among the mechanisms that have been implicated in the pro-carcinogenic effect of HCV infections, increased production of reactive oxygen species in the liver seems to have a major pathogenic role in the progression from chronic inflammation to cancer [72].

Recent data have also demonstrated that HCV is capable of inducing the active production of free radicals *per se*, not just through inflammation, a feature peculiar to this virus and the specific activity of its core protein [73]. Moreover, our results in rats indicated that one of the manifestations of the incidence of cancer is the formation of free radicals, which plays an important role in lipid peroxidation, liver damage, and carcinogenicity [28].

It is well-documented that *Panax ginseng* C.A. Meyer attenuates lipid peroxidation and is also capable of scavenging reactive oxygen species [28,74]. These

results support the hypothesis that ginseng has cancer preventive and antiviral effects, as suggested by the earlier animal experiments that suggested that Korean *Panax ginseng* had non-organ specific preventative effects against cancer [75].

In conclusion, the results of the current study indicate that KGE acts as liver cancer preventative as well as an antiviral agent against HCV. In HCV patients, the mechanism of KGE as an antiviral may be due to 1) its ability to increase cellular immunity, 2) its role in the scavenging free radicals generated by the virus, and 3) the increase in antioxidant enzymes in the cell. The mechanisms by which ginseng induces its anticancer effects include 1) its antioxidants properties, 2) an increase in antioxidant enzymes in the cell, 3) its free radicals scavenger properties, 4) an increase in the regeneration of liver cells, 5) a decrease in lipid peroxidation, and 6) inhibition of cytochrome P450 and/or activation of cytochrome P53.

ACKNOWLEDGMENTS

This work was fully supported by the grant (2007) from the Korean Society of Ginseng funded by Korea Ginseng Corporation (KGC). The capsule of KRG used in this study was supplied by KGC.

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