

Hepatic protective effect of grape seed proanthocyanidin extract against Gleevec-induced apoptosis, liver Injury and Ki67 alterations in rats

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Gleevec (imatinib) is an antineoplastic chemotherapeutic agent used in the treatment of many types of cancer. The current study was conducted to examine the possible modifying effects of grape seeds proanthocyanidins extract (GSPE) against apoptosis, liver injury and Ki67 alterations induced by Gleevec in male rats. 40 male albino rats were equally divided into four groups (First and second groups were control and GSPE groups; third group was Gleevec group and fourth group was treated with Gleevec and GSPE). Gleevec induced elevations in P53 and depletion of Bcl2 levels in liver tissues were compared with the control group. Liver sections in rats treated with Gleevec exhibited marked cellular infiltrations, vacuolar degeneration hepatocytes, numerous apoptotic cells, and congestion in central and portal veins, as well as a significant increase in the proliferating of Ki67 after Gleevec injection as compared with control group. In contrast, treatment with Gleevec and GSPE showed a moderate to good degree of improvement in hepatocytes with a significant increase in Ki67, a decrease in P53 and an increase in Bcl2 levels in liver tissues compared to treatment with Gleevec. Therefore, Gleevec induces apoptosis, injury and Ki67 changes in rat liver, whereas GSPE modulates these alternations.

Keywords: Chemotherapy. Gleevec/effects. Gleevec/Imatinib. Proanthocyanidins. Liver. Apoptosis. Proliferation.

INTRODUCTION

Chemotherapy has been used for cancer treatments where it destroys cancer cells or may stop cancer cells from spreading, slows the growth of cancer cells and may cause toxic effects in normal cells surrounded by cancer that can severely affect the life of patients, which may cause death (Tousson *et al.*, 2014a,b,c; 2016). Gleevec (imatinib) is an antineoplastic chemotherapeutic agent used in the treatment of chronic myelogenous leukemia, gastrointestinal stromal tumors, several other malignancies and hypereosinophilic syndrome (Cools *et al.*, 2003; Nakajima, Toga, 2003; Droogendijk *et al.*, 2006). The

most common side-effects with Gleevec are weight gain, neutropenia, thrombocytopenia, anemia, headache, nausea, vomiting, diarrhea, dyspepsia, abdominal pain, oedema, skin rashes, muscle spasm and cramps, muscle and joint pain, and fatigue (Gini, Hessenm Minnaard, 2005).

Recently, several polyphenolic antioxidants derived from grape seeds have been implicated in cell protection (Bagchi *et al.*, 2014; Zhu *et al.*, 2014). Grape seed proanthocyanidin extract (GSPE) is a rich source of proanthocyanidins that are natural antioxidants, which are composed of various polyphenolic compounds and generally believed to protect against reactive oxygen species (ROS)-mediated myocardial ischemia/reperfusion injury and apoptosis (Fan, Lou, 2004; Cuevas *et al.*, 2011; Zhu *et al.*, 2014). Proanthocyanidins, also called condensed tannins, are oligomers and polymers

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of monomeric flavonoids (flavan-3-ols), containing various amounts of catechin and epicatechin, widely distributed in the plant kingdom, and which appears in fruits, vegetables and seeds especially those extracted from grape seeds (Mouradov, Spangenberg, 2014; Malisch *et al.*, 2015). Proanthocyanidins have become of great interest due to their biological properties, such as antioxidant, anti-inflammatory and anticancerigen properties, with further investigation of interest due to the potential use of proanthocyanidins in cancer prevention, in addition to their protective effects by reducing mitochondria damage and inhibiting cell apoptosis (Zhou, Raffoul, 2012). Therefore, the present study was conducted to examine the possible modifying effects of GSPE against liver injury and Ki67 alterations induced by Gleevec in male rats.

MATERIAL AND METHODS

Chemicals and reagents

Gleevec: Imatinib (Unistin 50 mL/50 mg vial Eimc united Pharmaceutical Badr City, Cairo, Egypt).

Grape seeds proanthocyanidins: Sigma-Aldrich offers USP-1298208.

Experiments were performed on 40 male albino rats weighing 130-150 g and 9-10 weeks of age. The rats were kept in our Faculty animal house for one week before the experimental work, and were maintained on a standard rodent diet and water available *ad libitum*.

Animal treatments

After a week of acclimatization, the rats were equally divided into four groups:

1st group: The control group included rats that did not receive treatment.

2nd group: GSPE or a positive control group in which animals received orally GSPE by stomach tube a dose of 50 mg/kg body weight twice a week for four weeks according to Zhang *et al.* (2005). It was purchased from Sigma Company.

3rd group: Gleevec group in which rats were injected intraperitoneally with Gleevec (1 mg/kg body weight/ twice a week) (Unistin 50 mL/50 mg vial Eimc united Pharmaceutical Badr City, Cairo, Egypt) for four weeks according to Prasad, Ramnarayan and Bairy (2010).

4th group: Co-treated group included rats that received GSPE (50 mg/kg B.W/ twice a week) orally for four weeks

and injected simultaneously by Gleevec (1 mg/kg BW) twice a week for four weeks.

Determination of the P53 and Bcl2 protein levels

P53 protein was determined by Assay designs kit (catalog No.900-117) while Bcl-2 was determined by Assay designs kit package (catalog No.900-133). According to the manufacturer's instructions, determining P53 and Bcl2 protein levels was performed by colorimetric procedures using human ELISA kit (Biospes, Chongqing, china) according to Findley *et al.* (1997) and Binabaj *et al.* (2015).

Histopathological examination

At the end of the experimental period, animals were seen to have fasted overnight and immediately after decapitation, rats were dissected, and liver from different groups were quickly removed and fixed in 10% neutral buffered formalin. After fixation, specimens were dehydrated, cleared in xylene and embedded in molten paraffin. Sections of 5 μ m thickness were cut using a rotary microtome and stained with Ehrlich's haematoxylin and counterstained with eosin as a routine method after Bancroft and Stevens (1990).

Immunohistochemical detection of Ki67

The expression of Ki67 immunoreactivity (Ki67-ir) was detected using avidin Biotin Complex (ABC) method (Tousson *et al.*, 2015). The sections were incubated with anti-mouse Ki67 monoclonal antibody (dilution 1:50, DAKO Japan Co, Ltd, Tokyo, Japan) for 1-2 h at room temperature.

Statistical analysis

Data were expressed as mean values \pm SE and statistical analysis was performed using one way ANOVA to assess significant differences among treatment groups. The criterion for statistical significance was set at $p < 0.01$ for the biochemical data. All statistical analyses were performed using SPSS statistical version 16 software package (SPSS[®] Inc., USA).

RESULTS

Gleevec induced apoptosis

Table I shows the concentration of apoptotic protein P53 and anti-apoptotic protein Bcl2 in liver tissue of

TABLE I - The apoptotic protein (P53) and the antiapoptotic protein (Bcl₂) levels in rat liver tissues in different group

	Control	GSPE	GC	GC+ GSPE
P53 (pg/mL)	4.12 ± 0.117*	4.09 ± 0.134*	4.95 ± 0.190 [#]	4.22 ± 0.207 [#]
Bcl₂ (pg/mL)	3.562 ± 0.098*	3.655 ± 0.105*	3.126 ± 0.119 [#]	3.490 ± 0.284 [#]

Significant difference from the control group at **p*<0.01. Significant difference from Gleevec group (GC) at [#]*p*<0.01; Where proanthocyanidins (GSPE), Gleevec (GC) and proanthocyanidins plus Gleevec (GSPE+GC).

different groups. P53 levels were significantly increased in treated rats with Gleevec when compared with control group. In contrast, prophylactic treatment with Gleevec and GSPE prevented Gleevec induced elevations in P53 levels compared to the control group (Table I). On the other hand, the anti-apoptotic protein Bcl2 levels in liver tissue were significantly (*P*<0.01) decreased in Gleevec when compared with control group; while, prophylactic treatment with Gleevec and GSPE prevented Gleevec induced depletion in Bcl2 levels compared to the control group (Table I).

Effect of GSPE on liver histopathology

Liver sections of control and GSPE groups exhibited

the normal architecture of the hepatocytes with prominent round, vesicular basophilic nuclei and eosinophilic cytoplasm, and a few spaced hepatic sinusoids arranged in-between the hepatic cords (Figure 1A and B). Liver sections of Gleevec group exhibited marked cellular infiltrations, moderate atrophied vacuolar degeneration hepatocytes, numerous apoptotic cells with deep eosinophilic cytoplasm and small deeply stained pyknotic or fragmented nuclei, as well as marked dilation or congestion in central and portal veins (Figure 1C). Liver sections in treated Gleevec with GSPE exhibited a mild degree of improvement in hepatocytes that exhibits moderate vacuolated hepatocytes with mild cellular infiltrations and moderate congestion in central and portal veins (Figure 1D).

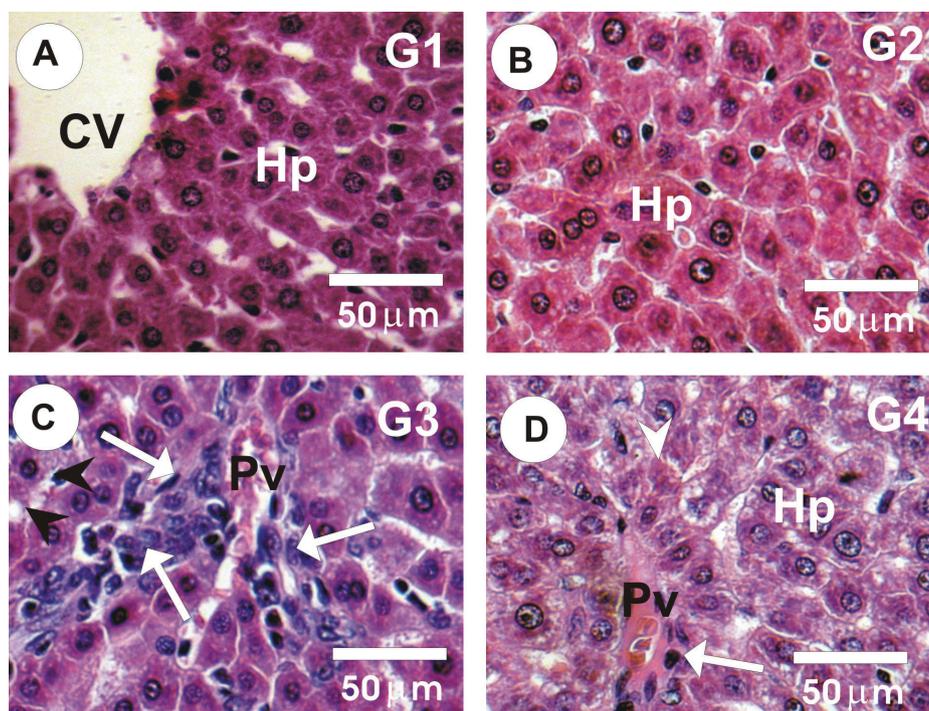


FIGURE 1 - Photomicrographs of rat liver sections stained by HE; (A&B) Rat testes in control (G1) and GSPE (G2) groups showed normal architecture of the hepatocytes (hp) with central vien (CV); (C) Liver sections of Gleevec group (G3) exhibited marked cellular infiltrations (White arrows), moderate atrophied vacuolar degeneration hepatocytes (arrow heads), and many of apoptotic cells; (D) Liver sections treated Gleevec with GSPE (G4) exhibited a mild degree of improvement in hepatocytes that exhibits moderate vacuolated hepatocytes (arrow heads) with mild cellular infiltrations (White arrows) and moderate congestion in central and portal veins.

Effect of GSPE on liver Ki67 immunohistochemical changes

The detection and distribution in Ki67 immunoreactivity (Ki67-ir) in liver sections in the different groups are shown in Figures 2A-2D. Strong positive reaction for Ki67-ir (grade 4) in hepatocyte nuclei was observed in liver section in control and GSPE groups (Figures 2A and B). In contrast, mild to faint positive reactions were detected for Ki67-ir (grade 1) in the liver sections in Gleevec rats group (Figure 2C). On the other hand, moderate positive reactions for Ki67-ir (grade 2) were observed in liver sections of treated Gleevec with GSPE (Figure 2D).

DISCUSSION

Chemotherapy involves the use of chemical agents to stop the growth and elimination of cancer cells even at distant sites from the origin of primary tumor. However, chemotherapy does not distinguish between cancer and normal cells, and eliminates not only the fast-growing cancer cells but also other fast-growing cells in the body, including blood cells. Although a number of studies have

demonstrated some side-effects of the chemotherapeutic drugs, the current work is aimed at studying the possible modifying effects of GSPE extract against liver injury, apoptosis and changes in proliferating markers Ki67 induced by Gleevec in the liver tissues in male rats.

When cells are exposed to external damage stimuli or injury, they activate the regulation of expression of these genes. P53 tumor suppressor protein is a transcription factor that regulates the DNA repair and apoptosis, while Bcl-2 gene is an anti-apoptotic protein, which has important roles in regulating cell survival (Tousson *et al.*, 2014a; 2016). An inverse correlation was found between the expression of Bcl-2 and p53 proteins in our results. In the current study, injection with Gleevec for four weeks induced elevations in P53 and depletion in Bcl2 levels in liver tissues compared to the control group. In contrast, treatment of rats with Gleevec and GSPE significantly decreased the P53 and increased the Bcl2 levels when compared with Gleevec group. The results from study agreed with Tousson *et al.* (2014a; 2016) who reported that Amethopetrin induced apoptosis in lung and heart rats and L-carnitine treatment improved these alternations.

In the current study, a moderate atrophied vacuolar degeneration hepatocytes, marked cellular infiltrations,

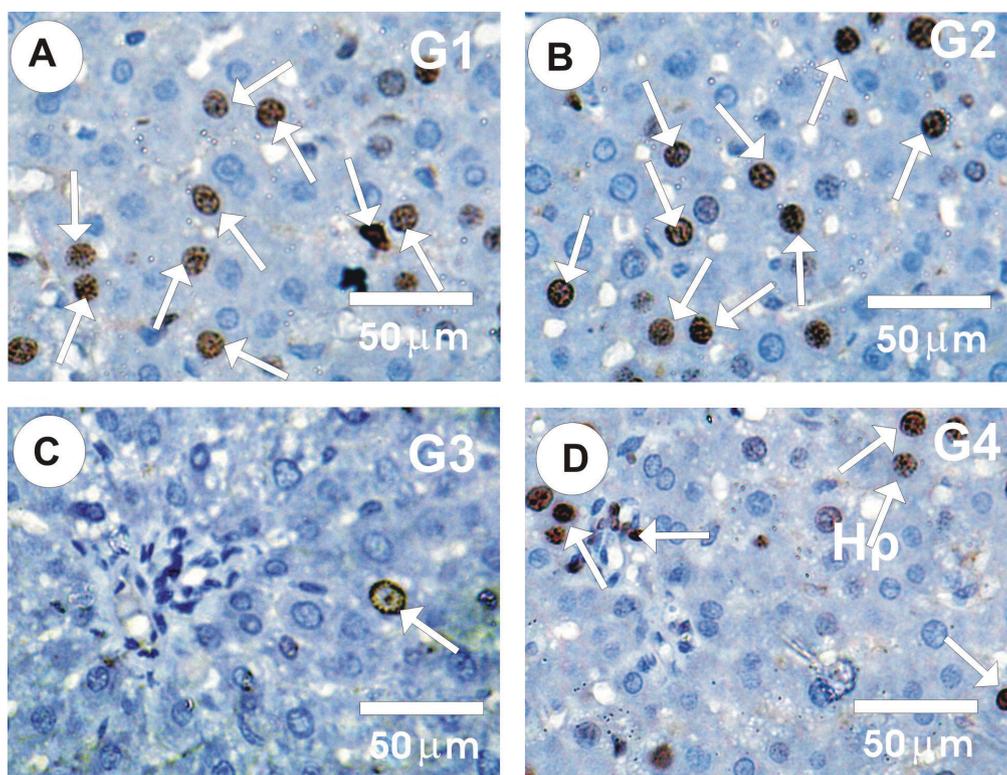


FIGURE 2 - Photomicrographs of liver sections stained with KI67-ir in the different groups; **(A&B)** Liver section in control and GSPE groups showed strong positive reaction (arrows) for Ki67-ir (grade 4) in hepatocyte nuclei; **(C)** Liver sections in Gleevec rats group showed mild to faint positive reactions for Ki67-ir; **(D)** Liver sections in treated Gleevec with GSPE showed moderate positive reactions (arrows) for Ki67-ir.

and marked dilation or congestion in central and portal veins were observed in liver sections after Gleevec injection. Our histopathological results showed that treatment of rat liver with GSPE showed a mild degree of improvement in hepatocytes in liver sections. Our results agree with Kart *et al.* (2010) and Basuony *et al.* (2015) who reported that chemotherapy drugs induces hepatotoxicity, oxidative injury and cellular abnormalities in liver. Similar findings were reported by El-Sayyad *et al.* (2009) and Abdel-Wahhab *et al.* (2014) who revealed that many hepatocytes showed marked degeneration in hepatic cords in addition to karyomegaly and pyknotic nuclei indicating apoptosis after treatment with chemotherapy.

The most widely used proliferation-associated marker is Ki-67, which is a nuclear antigen present only in proliferating cells (Lebe *et al.*, 2007). In the current study, a significant decrease in Ki67 was observed in liver sections after Gleevec injection when compared with control. This current result is in harmony with Leitão *et al.* (2011) who reported that MTX treatment significantly decreased the numbers of Ki-67-positive cells. Although the numbers of Ki-67-positive cells had a significant decrease in the Gleevec group in the present study, this decrease was not observed in the co-treated Gleevec with GSPE groups.

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COMPETING INTEREST

Authors have declared that no competing interest exists.

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