Annual Research & Review in Biology

20(6): 1-9, 2017; Article no.ARRB.37766
ISSN: 2347-565X, NLM ID: 101632869

Protective Potential of Grape Seed Proanthocyandins Extract against Glivec (Imatinib Mesylate) Induced Liver Toxicity and Oxidative Stress in Male Rats

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2017/37766
Editor(s):
(1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Filip Nina, University of Medicine and Pharmacy Grigore T. Popa Iasi, Romania.
(2) Amal A. E. Ibrahim, Ain Shams University, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/22268

Original Research Article

Received 28th October 2017
Accepted 4th December 2017
Published 13th December 2017

ABSTRACT

Objectives: Glivec (Imatinib mesylate) an antineoplastic chemotherapeutic agent used in the treatment of many types of cancer. The current study examines the hepatoprotective potential of grape seed proanthocyandins extract (GSPE) against Glivec induced oxidative stress and toxicity in male albino rats.
Materials and Methods: A total of 40 male albino rats were equally divided into four groups; group 1 was control, group 2 was GSPE group (rats received orally GSPE by stomach tube {50 mg/kg

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BW/twice a week} for four week), group 3 was Glivec group (rats were injected intraperitoneally with Glivec {1 mg /kg B W/twice a week} for four weeks) and group 4 was rats treated with GSPE plus Glivec for four weeks.
Results: LD50 was calculated for Glivec in rats (estimated at 598 mg/kg), presenting confidence limits between 588 and 612 mg/kg body weight. A significant increase in the liver TBARS and aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and γ-glutamyltransferase (GGT) activities in Glivec group when compared with the control group. On the other hand; a significant decrease in the serum albumin, globulin, total protein, liver superoxide dismutase activity (SOD), catalase (CAT), glutathione S-trasferase (GST) and reduced glutathione (GSH) levels in Glivec group when compared with the control group. Administration of GSPE with Glivec caused a protective and ameliorative effect against Glivec induced liver toxicity. **Conclusions:** Treatment with GSPE has a promising role for ameliorating the oxidative stress and hepatic injury induced by Glivec.

Keywords: Chemotherapy; Imatinib mesylate; Glivec; GSPE; liver; oxidative stress.

1. INTRODUCTION

Cancer cells differ from normal cells in many ways that allow them to grow out of control and become invasive. Cancer is caused usually due to abnormalities in the DNA of the affected cells leading to an extra mass of tissue called a tumor [1]. Most of chemotherapy that used for cancer treatments can severely affect the life of patients and represent a direct cause of death [2-8].

Glivec is the trade name for the generic drug name imatinib mesylate an antineoplastic chemotherapeutic agent that used in the treatment of many types of cancer [9,10]. Glivec-induced hepato-toxicity in humans [10-12]. Glivec can cause many different types of symptoms as weight increase, abdominal pain, muscle spasm and cramps, diarrhoea, vomiting, neutropenia, thrombocytopenia and headache [13].

Many plant extracts and their products have been shown to have significant antioxidant activity which may be an important property of medicinal plants associated with the treatment of several ill-fated diseases including liver toxicity [8,14]. Proanthocyanidins (tannins) are high-molecular weight polymers comprised of the monomeric flavonoids (flavan-3-ols) contain various amounts of catechin and epicatechin, widely distributed in the plant kingdom, appearing in fruits, vegetables and seeds [15]. Grape seed is rich sources of proanthocyanidins that are natural antioxidants composed of various polyphenolic compounds and have been demonstrated to exhibit a broad spectrum of pharmacological, therapeutic and chemo-protective properties [16-18]. The natural extracts of grape seed are containing approximately 89% proanthocyanidins, with dimmers (6.6%), trimers (5.0%), tetramers (2.9%) and oligomers (74.8%), as described previously [19].

Grape seed proanthocyanidins (GSP) have become of high interest because of their biological properties, such as anti-inflammatory and anticancerigen properties with further investigation of interest due to proanthocyanidins potential use in cancer prevention in addition to its protective effects by reducing mitochondria damage and inhibiting cell apoptosis [18,20,21]. In addition to GSP are attributed to the antioxidant activity, prevents ROS-induced DNA damage and possesses strong free radical scavenging activity, which provides excellent protection against oxidative stress and free radical-mediated tissue injury under pathological conditions [22]. Therefore; the present study was conducted to examine the possible modifying effects of grape seeds proanthocyandins aqueous extract (GSPE) to prevent oxidative stress and toxicity of Glivec in rat liver.

2. MATERIALS AND METHODS

2.1 Chemical and Reagent

Glivec® (400 mg; Imatinib mesylate) was obtained from Novartis Pharmaceuticals Corporation Basel, Switzerland.

Grape seed proanthocyandins was obtained a dried powdered grape seed proanthocyanidin extract (GSPE) commercially known as Noxy life was obtained from Pharco Pharmaceuticals (Mansoura, Egypt).

2.2 Animals

The experiments were performed on 40 male albino rats weighing 130-150 g and 10-11 weeks old were purchased from the breeding unit of Egyptian Organization for Biological Products and Vaccines (EOBPV), Abbassia, Cairo. The animals were housed in steel mesh cages and maintained for one week acclimatization period on commercial standard and pellet diet and drinking water *ad libitum*. The housing cycle was 12:12 h light/dark cycle under controlled temperature (20-22ºC). The animal use protocol had been approved by the Institutional Animals Ethics Committee (IAEC) of Tanta University.

2.3 Experimental Design and Treatment

After one weeks of acclimation, rats were equally divided into four groups. 1st group, control group included rats received no treatment; 2nd group, GSPE group in which animals received orally GSPE by stomach tube (50 mg/kg BW/twice a week) for four week according to Zhang et al. [23]; 3rd group, Glivec group in which rats were injected intraperitoneally with Glivec (1 mg /kg body weight/twice a week) for four weeks according to Prasad et al. [24]; 4th group, treated rats with GSPE (50 mg/kg B.W/twice a week) plus Glivec (1 mg/kg BW/twice a week) for four weeks.

2.4 Determination of Serum Enzymes

At the end of the experimental period, animals were fasted overnight and for clinical chemistry, blood samples were individually collected from the inferior vena cava of each rat in non-heparinized glass and allowed to stand for 30 min at room temperature to clot before being centrifuged at 3000×g for 15 min.

Both alanine transaminase (ALT) and aspartate transaminase (AST) activities in serum were assayed by using commercial kit that was supplied by Humann (Germany) according to the method of Schumann and Klauke [25]; alkaline phosphatase (ALP) activity in serum was assayed by using commercial kit that was supplied by Humann (Germany) according to the method of Moss and Henderson [26]. γ Glutamyl transferase (GGT) activity in serum was assayed by using commercial kit that was supplied by Randox (United Kingdom) according to the method of Johnston [27]. Globulins activity in serum was assayed by using commercial kit that was supplied by Human Diagnostic, Germany according to the method of Johnston [26]; albumin concentration in serum was assayed by using commercial kit that was supplied by Diamond (Egypt) according to the method of Doumas et al. [28].

Total bilirubin activity in serum was assayed by using commercial kit that was supplied by Randox (United Kingdom) according to the method of Aman et al. [29]. Total protein concentration was determined spectrophotometrically using commercial diagnostic kits supplied by Diamond (Egypt) according to the method of Bowers and Wong [30].

2.5 Preparation of Liver Homogenates

At the end of the experimental period, rats from each group were euthanized with anesthetic ether and subjected to a complete necropsy after 10–12 h of fasting. After animals were sacrificed, the liver was instantly removed, washed three times in ice cold saline and blotted on ash free filter paper, then used for preparation of tissue homogenates for estimation of tissue TBARS and GSH levels and the activity of SOD, GPx, GST and CAT enzymes. Liver homogenates were prepared as reported by Sakeran et al. [31]. Briefly, Liver tissue from each rat were separated, weighed and homogenized separately with a Potter Elvenhjem tissue homogenizer. The crude tissue homogenate was centrifuged at 10,000 g for 20 min at 4°C, and the resultant supernatant was used for the different enzyme assays.

2.6 Enzymatic and Non-Enzymatic Antioxidant Assays

Thiobarbituric acid-reactive substances (TBARS) were measured in homogenate using the method of Sakeran et al. [31].

Reduced glutathione (GSH) content was measured after reaction with 5,5′- dithiobis-(2-nitrobenzoic acid) using the method of Ellman [32].

Superoxide dismutase activity (SOD; EC 1.15.1.1) was determined according to Misra and Fridovich [33]. The assay procedure involves the inhibition of epinephrine auto-oxidation in an alkaline medium to adrenochrome, which is markedly inhibited by the presence of SOD.

The enzyme catalase (CAT; EC 1.11.1.6) converts H_2O_2 into water. The CAT activity was measured spectrophotometrically at 240 nm by calculating the rate of degradation of H_2O_2, the substrate of the enzyme [34].

Glutathione *S*-transferase (GST; EC 2.5.1.18) activity was determined according to Habig et al. [35] using para-nitrobenzyl chloride as a substrate. The glutathione peroxidase (GPx, EC 1.11.1.9) activity was analyzed according to the method described by Hafeman et al. [36]. GPx degrades H_2O_2 in the presence of GSH thereby depleting it. The remaining GSH is then measured by using 5,5′- dithiobis-(2-nitrobenzoic acid).

2.7 Statistical Analysis

Data were expressed as mean values ± 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 21 software package (SPSS® Inc., USA).

3. RESULTS

3.1 Toxicity

No mortality was observed in control or in GSPE rat groups during the period of the study.

The dose of GSPE did not initiate any side effects for the rats. On the other hand; a total of 13.3 ± 0.65% mortality was recorded in Glivec group, while 6.6±1.1% mortality was recorded in treated of Glivec with GSPE group.

LD50 was calculated for Glivec in rats (estimated at 598 mg/kg), presenting confidence limits between 588 and 612 mg/kg body weight.

In addition to; various side effects were observed in rats after the injections of Glivec as in the 3rd and 4th groups such as yellowish body hair, weakness and loss of activity.

3.2 Effect of GSPE on Hepatic Biochemical Markers

The data summarized in Table 1 indicates that a significant ($P<0.01$) elevation in ALT, AST, ALP and GGT in Glivec group when compared with control group, this elevations significantly ($P<0.01$) decreased after the treated of Glivec with GSPE. On the other hand; albumin, globulin and total protein were significantly ($P<0.01$) decreased in Glivec group when compared with control group, this depletion significantly ($P<0.01$) increased after the treated of Glivec with GSPE (Table 1). In contrast; total Bilirubin levels showed insignificant decrease in Glivec group when compared with control group, while the treatment of rats with Glivec and GSPE recovered the total Bilirubin levels to normal levels (Table 1).

3.3 Lipid Peroxidation and Reduced Glutathione Content

Table 2 shows a significant ($P< 0.01$) increase in TBARS content, the indicator of LPO in liver after Glivec treatment. The levels of LPO were increased as compared with control while rats treated with both Glivec and GSPE showed a significant decrease in TBARS level as compared with Glivec treated rats. On the other hand, GSH content was significantly ($P< 0.01$) decreased in Glivec treated rats as compared with control (Fig. 1B). In contrast; rats treated with both Glivec and GSPE showed significant ($P< 0.01$) increase in GSH content as compared with Glivec treated rats (Table 2).

3.4 Antioxidant Enzyme Activities

The data summarized in Fig. (1A-1D) indicates that a significant ($P<0.01$) depletion in liver SOD, CAT, GPx and GST in Glivec group when compared with control group. On the other hand, rats treated with Glivec and GSPE showed significant ($P<0.01$) alleviation in the antioxidant enzyme activities as compared with Glivec treated ones (Fig. 1A-1D).

4. DISCUSSION

The current study aimed to study the possible modifying effects of GSPE against liver toxicity induced by Glivec in male albino rats. Glivec (Imatinib mesylate)-induced hepatic toxicity in humans [11,12]. Many side effects were detected in humans after the use of Glivec as neutropenia, thrombocytopenia and anaemia [13]. Tonyali et al. [37] who reported that; Imatinib mesylate-induced acute liver failure in a patient with gastrointestinal stromal tumors. DeLeve [10] and Shah et al. [38] who reported that; the uses of Glivec-induced liver toxicity in humans. Glivec is associated with three forms of acute liver injury,

Table 1. Enzyme activities and biochemical parameters in serum of male rats in different groups

Parameters	Control	GSPE	GC	GC+GSPE
AST (U/l)	24.29±0.847*	23.48± 1.556*	87.19±4.234$^{\#}$	39.75±1.68*$^{\#}$
ALT (U/l)	118.55±7.580*	114.60±9.255*	187.24±5.672$^{\#}$	125.45±11.117*
ALP (U/l)	108.5±7.445*	102.5±6.824*	193.2±11.035$^{\#}$	151.6 ±9.117*$^{\#}$
GGT (U/l)	61.9±4.18*	56.4±3.28*	90.4±5.35$^{\#}$	73.5 ±5.38*$^{\#}$
Globulin (g/dl)	4.74±0.226*	4.78±0.357*	3.51±0.208$^{\#}$	4.59±0.315*
Albumin (g/dl)	3.75 ±0.154*	3.81±0.187*	3.30±0.159$^{\#}$	3.54±0.205*$^{\#}$
T. Bilirubin (g/dl)	0.26 ±0.15	0.25 ±0.09	0.26 ±0.14	0.26 ±0.21
Total protein (g/dl)	7.15±.285*	6.92 ±0.422*	6.23±0.413$^{\#}$	6.49±0.356$^{\#}$

*Values are expressed as means ± SE; n = 6 for each treatment group. Significant difference from the control group at *p<0.01. Significant difference from Glivec group (GC) at $^{\#}$p<0.01; Where grape seeds proanthocyandins extract (GSPE), Glivec (GC)*

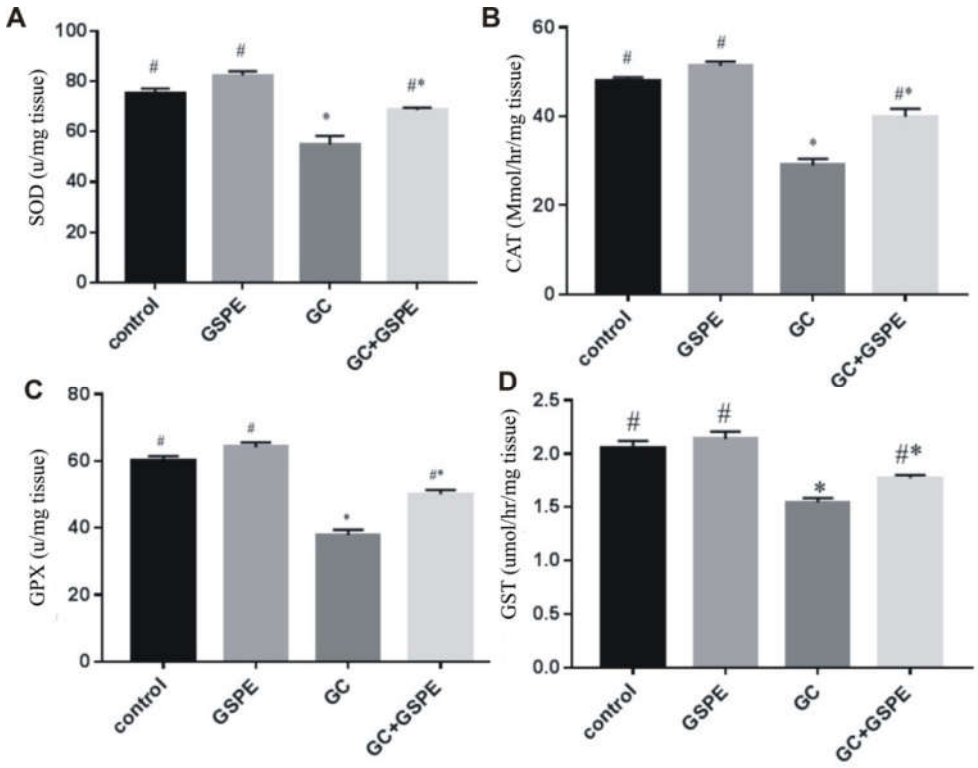

Fig. 1. Effect of proanthocyandins extract treatment on liver enzymatic antioxidants SOD (A), CAT (B), GPx (C) and GST (D) activity in Glevic induced hepatotoxicity in experimental rats

*Bar represents mean±SE. N=6 animals per group. Significant difference from the control group at *p<0.01. Significant difference from Glivec group (GC) at $^{\#}$p<0.01; Where grape seeds proanthocyandins extract (GSPE), Glivec (GC)*

this forms is transient and usually asymptomatic elevations in serum enzymes during treatment, clinically apparent acute hepatitis, and reactivation of an underlying chronic hepatitis B. Chemotherapy-induced hepatotoxicity is a common cause of abnormal liver function test in patients, this hepatotoxicity are usually begins with vague clinical symptoms such as fatigue, anorexia, nausea, dark urine, right upper quadrant discomfort and jaundice.

In the current study; a significant increase in ALT, AST, ALP and GGT in Glivec group however, this elevation decreased in treated group with GSPE. On the other hand; depletion were detected in albumin, globulin and total

Table 2. Changes in thiobarbituric acid-reactive substances (TBARS) and reduced glutathione (GSH) in rat liver in different groups

Parameters	Control	GSPE	GC	GC+GSPE
TBARS (nmol/g tissue)	33.095±1.35*	31.985±1.02*	49.622±2.46#	36.220±1.71*#
GSH (mmol/mg protein)	2.435±0.023*	2.681±0.09*	1.594±0.11#	2.044±0.18*#

*Values are expressed as means ± SE; n = 6 for each treatment group. Significant difference from the control group at *p<0.01. Significant difference from Glivec group (GC) at #p<0.01; Where grape seeds proanthocyandins extract (GSPE), Glivec (GC)*

protein in Glivec group. Elevated levels of serum AST and ALT enzymes are indicative of cellular leakage and loss of functional integrity of cell membranes in the liver [39]. In contrast; the reduction in liver proteins caused by Glivec could be attributed to the damaging effect of Glivec on liver cells.

Similar findings were reported by Abouzeinab [2]; Nasr [40]; Abdel-Wahhab et al. [41] and Basuony et al. [42] who reported that cisplatin induced hepatotoxicity in male rats. The current results agreed with Juma [43] and McDonald et al. [44] who reported that; cyclophosphamide induced toxicity in human liver. Also; these results are in agreement with the previous findings of Kamboj and Sandhir [45] and Valentovic et al. [46] who observed a significant decline in serum biochemical parameters due to cisplatin treatments.

The increase in concentration of total protein and their fractions within the normal range in control and GSPE groups may reflect the normal levels in the hepatic function while the decrease in concentration of total protein and their fractions in Glivec group may reflect the toxicity in the liver. These findings suggest that the treatment of Glivec with GSPE may increase the metabolic rate. It is known that the change in albumin level reflects the change in liver function and the presence of the fatty acids may affect muscle protein synthesis and protein deposition through a prostaglandin-dependent mechanism. These results agree with Basuony et al. [42] who reported that Cisplatin increase the liver functions were decrease serum albumin and total proteins in male rats.

This current result is in harmony with Tousson et al. [5,6] who reported that; methotrexate-induced hepatic and renal toxicity in male rats and the increased in liver function associated with free radicals trigger cell damage through binding to cellular macromolecules. There was a significant (P<0.01) restoration of these enzyme levels on administration of the GSPE as co-treatment. The reversal of increased serum enzymes in Glivec induced liver damage by the GSPE may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes after rosemary and rosmarinic acid treatment on acetaminophen-induced liver damage.

In the current study; a significant increase in liver TBARS in Glivec group however, this elevation decreased in treated group with GSPE. On the other hand; a significant decrease in liver GSH, SOD, CAT, GPx and GST in Glivec group however, this depletion decreased in treated group with GSPE. Similar findings were reported by Zarei and Shivanandappa [47] who reported that; cyclophosphamide-induced oxidative stress and hepatotoxicity in mice. Also; or results agree with Kart et al. [48] who reported that, cisplatin-induced hepatotoxicity and oxidative stress in rabbit liver. In agreement with our results; Valentovic et al. [46] who reported that the decrease in the SOD and GPx activities and increase in LPO could explain the induction of free radicals in cisplatin-treated rats.

Zarei and Shivanandappa [47] and Tousson et al. [4] showed that antioxidant can be useful in preventing hepatocellular damage by inhibiting lipid peroxidation by free radicals generated by Chemotherapy. GSP are highly efficient natural antioxidants, their antioxidant activity is 50 times higher than that of vitamin E and 20 times that of vitamin C, and their effects have been reported in a wide range of studies [18]. Proanthocyanidins is known to have hepatoprotective and anticarcinogenic effects in addition to its protective effects by reducing mitochondria damage and this ability of Proanthocyanidins leads to a significant increase in the cellular antioxidant defense machinery by ameliorating the deleterious effects of free radical reaction and by the increase in GSH content, which is

important in maintaining the ferrous state [18,20,21].

From our data, it was shown that the mice co-treated with GSPE saw increased levels of reduced GSH, SOD, CAT, GPx and GST and decreased the level of TBARS, a result that might indicate that GSPE could directly reduce phospholipid hydroperoxides within the membrane and lipoproteins by removing the ROS' ability to inhibit lipid peroxidation of liver cells induced by Glivec. Recent results of the meta-analysis by Li et al. [18] indicated that GSP could effectively improve the activity of anti-oxidative enzymes and reduce lipid peroxidation products. Using GSPE in combination with Glivec minimized and alleviated its hazardous effects on most of the tested parameters and this may be attributed to the vital role of GSPE as antioxidant. In general, treatment with GSPE alone improved the antioxidant status of rats and could be useful as antioxidant against stress induced by chemotherapy.

5. CONCLUSION

Glivec (Imatinib mesylate)-induced hepatic toxicity, oxidative stress. Administration of grape seed Proanthocyanidins extract with Glivec caused a protective and ameliorative effect against Glivec induced liver toxicity and oxidative stress and hepatic injury induced by Glivec.

ACKNOWLEDGEMENTS

This work was supported by the Deanship of Scientific Research (DSR), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia, under grant No.37-K-180. The authors therefore, gratefully acknowledge the DSR technical and financial support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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