The Pharma Innovation Journal 2019; 8(6): 282-285

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(6): 282-285
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www.thepharmajournal.com
Received: 24-03-2019
Accepted: 28-04-2019

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# Hematological and serum biochemical evaluation of imatinib mesylate induced toxicity in wistar rats and its amelioration with grape seed proanthocyanidins

**Immanni Hemanth, HD Narayanaswamy, ML Satyanarayana, Suguna Rao and Prakash Nadoor**

**Abstract**
Non-target organ toxicities has been a critical issue in the clinical management of various neoplasm through chemotherapeutic agents. Imatinib mesylate, the first molecule among Tyrosine kinase inhibitor class of drugs has also been associated with hematological and various organ toxicities among human and animal patients. Grape seed proanthocyanidins on other hand was attributed with the protective abilities against various drug induced organ toxicities. The present study was conducted to investigate the ameliorative efficacy of GSPs in imatinib induced toxicity, through evaluation of hematological and serum biochemical parameters in male Wistar rats. The results indicated statistically significant ($P<0.05$) decrease in hematological values (TEC, Hb conc., PCV and TLC) and significant increase ($P<0.05$) in serum biochemical values (AST, ALT, ALP, Total protein, Creatinine and LDH) among drug control animals compared to normal ones. GSPs mediated amelioration was evident with significantly improved hematological and serum biochemical values among the animals co-administered with both imatinib and GSP extract, suggesting the chemoprotective efficiency of GSPs against imatinib mesylate induced non-target toxicity.

**Keywords:** Grape seed proanthocyanidins, hematology, imatinib mesylate, serum biochemistry, rats

## 1. Introduction
Chemotherapy has become an important treatment modality in cancer management during the recent times, both in human and veterinary medicine. Various new classes of chemotherapeutic agents have made their way in to the market with promising results of cancer treatment and extending patient survival [1].

Imatinib mesylate (Imb) is the novel drug belonging to the class of Tyrosine kinase inhibitor (TKIs), was introduced primarily for the treatment of chronic myeloid leukemia (CML) in humans [2]. Later, its clinical relevance has also been observed in the treatment of gastrointestinal stromal tumors (GISTs), melanoma, hemangiosarcoma, mast cell tumors, fibrosarcoma, squamous cell carcinoma e.t.c. in canine and feline patients [3, 4, 5]. However, non-target organ toxicity is the un-avoidable side-effect with Imb, as like other chemotherapeutic protocols. Specifically, its cardio-toxicity effects were demonstrated in both *in vitro* and *in vivo* experiments, with the proven mechanisms of free radical generation, mitochondrial function disruption, activation of endoplasmic reticulum stress response and initiation of apoptotic pathways [6]. In addition, it has also been implicated with the toxic effects in liver and kidneys [7, 8], by interrupting critical signaling pathways, suggesting the need for certain chemoprotective agents to counteract imatinib induced toxicities during the treatment of CML, GISTs etc.

Proanthocyanidins are naturally occurring polyphenolic compounds, widely available in fruits, vegetables, seeds, nuts, flowers and bark in plant kingdom [9]. Chemically, they are oligomers and polymers of monomeric flavonoids (flavan-3-ols), containing various amounts of catechins and epicatechins. Grape seed proanthocyanidins (GSPs) are one of the extensively investigated phytochemicals in the recent years, with proven mechanisms of biological, pharmacological, therapeutic, and chemoprotective properties against oxygen free radicals and oxidative stress [9,10]. In addition, their beneficial properties have been demonstrated in counteracting various drug and chemical-induced organ toxicities, in various experimental studies [11, 12].

Thus, the present study has been taken to assess the ameliorative effects of GSPs in Imb induced toxicity through hematological and serum biochemical evaluation in male Wistar rats.

## 2. Material and Methods

### 2.1 Drug and GSP extract procurement

Active pharmaceutical ingredient of Imb was procured from M/S. Vibgyor Drugs Pvt. Ltd., Hyderabad (Secunderabad) and GSP extract (95% proanthocyanidins) was procured from M/S. Natural Holistics, Bangalore.

### 2.2 Experimental Study

The study was carried out in Wistar male rats adhering to the guideline of Committee for the purpose of control and supervision of experiments on animals (CPCSEA), with approval from Institutional Animal Ethics Committee (IAEC). Male wistar rats of 6-8 weeks of age, with about 180-200 gm body weight were procured from M/S. Vivon biotech, Bangalore. They were housed in standard poly-propylene rat cages at 25±1°C room temperature, with 12 hour interval light/dark cycles, and provided with standard pellet feed *adlibitum*, throughout the experimental period of 28 days.

### 2.3 Experimental design

Upon acclimatization for a period of 10 days, rats were segregated into four experimental groups with 12 rats each.

**Group I:** Normal control rats with daily oral gavaging of distilled water.

**Group II:** Drug positive control rats with daily oral gavaging of Imb in distilled water @ 100mg/Kg. B.Wt.

**Group III:** GSP positive control rats with daily oral gavaging of GSP extract in distilled water @ 200mg/Kg. B.Wt.

**Group IV:** Treatment rats with concurrent oral gavaging with Imb @100mg/Kg. B.Wt. and GSP extract @ 200mg/Kg. B.Wt in distilled water, with 3-4 hour interval.

Six rats from each group were sacrificed on $14^{th}$ and $28^{th}$ Days under chloroform over-dose euthanasia.

### 2.4 Hematology

Whole blood was collected in 10% EDTA vials, from each animal during the sacrifice. Hematological parameters like Total erythrocyte count, Hemoglobin concentration, Hematocrit (PCV) and Total leucocyte count were analyzed using Mindray BC-2800Vet automatic haematology analyzer.

### 2.5 Serum biochemistry

Serum was obtained by collecting the whole blood into a sterile tube during sacrifice. Upon clotting, clear serum was separated by centrifuging at 3000 rpm for 5 minutes, without hemolysis and RBCs contamination and stored at $-20^0$C till further analysis. Serum biochemical parameters like AST, ALT, ALP, Total Protein, Creatinine and LDH were estimated through Labmate semi-automatic analyser, using commercial biochemical kits from M/S. Erba Mannheim diagnostics, Transasia Biomedicals Ltd.

### 2.6 Statistical analysis

The data were statistically evaluated with SPSS 21.0 software, through one-way analysis of variance (ANOVA) followed by Duncan's post hoc analysis. p-values less than 0.05 was considered as statistical significance.

## 3. Results and Discussion

The haematological values *viz.* TEC, Hb concentration, hematocrit and TLC values of the animals sacrificed on Days 14 and 28 of the present study was presented in Table 1. Among the Imb control animals, values pertaining to all parameters were found to be significantly lower ($P<0.05$) than the negative control rats. In contrast, the values in concurrently treated Group IV animals were found to be significantly higher ($P<0.05$), than Imb treated rats; but were significantly lower than negative control rats. However, there observed no significant difference between normal control rats and GSP control rats (Group III) on both the days of sacrifice. These alterations in the hematological profile could be attributed to toxic effect of Imb on the hematopoietic activity in Group II animals. This was in agreement with the earlier reports that suggested the hematological abnormalities upon Imb administration in rats, dogs and humans [5, 13, 14]. However, amelioration effect of GSP extract was observed among Group IV animals with the significantly increased hematological parameters, in comparison with Imb control animals.

Serum biochemical evaluation pertaining to the hepatic, renal and cardiotoxicity administration revealed significant abnormalities among Imb control rats in comparison with negative control animals (Table 2). There were a significantly increased levels of AST, ALT and ALP in Group II animals, indicating the hepatic injury subsequent to toxicity. However, there observed no significant difference in total protein levels among different treatment groups. In addition, serum levels of creatinine and LDH were also found to be significantly increased among Group II animals, indicating the renal and cardiac toxicities. Concurrently administered animals (Group IV) had revealed amelioration activity of GSPs with significantly improved serum biochemical values, than in Group II rats. These findings had indicated the Imb induced hepatic, renal and cardiac-toxicities in Group II animals, the mechanism of which were elucidated earlier.

In heart, the principal mechanism of cardiac myocytes toxicity is through free radical generation and subsequent oxidative stress, resulting in disruption of mitochondrial activity and cell damage, with subsequent release of cardiac specific enzymes like LDH and CPK into the serum [15, 16].

Imb induced liver toxicity was well established, especially in human patients under GISTs and CML therapy that ranges from mild, reversible and transient raise in liver non-functional enzymes to acute hepatic liver [17]. The suggested mechanisms of hepatic toxicity were oxidative stress, non-specific inhibition of other tyrosine kinases besides BCR-ABL within the hepatocytes along with drug-induced hypersensitivity and immuno-allergic reactions [18]. The ameliorative effect of GSPs might be due to certain molecular mechanisms like GSP induced expression of Ki67, Bcl2, and reduced levels of P53 that confers cellular protection and cell survival [7].

In kidneys, the proliferation and subsequent regeneration of proximal tubular cells depend on the activation of PDGFR signaling in the glomerulus, arteries, tubules, and interstitium, especially after acute tubular necrosis [19]. Since, Imb inhibits PDGFR pathways, in addition to the BCR-ABL, it may interfere with regular repair mechanisms of tubular epithelium resulting in tubular necrosis, with the elevated serum creatinine levels [20, 21]. In addition, a recent retrospective investigation of renal function in CML patients on imatinib therapy had suggested a blunting of tubular creatinine

secretion by Imb [22]. The proposed mechanisms of GSP mediated renal protection is through prevention of oxidative stress induced cell injury and through general protection of tubular epithelial cells as evident from earlier studies with metal toxicities [23].

**Table 1:** Mean±SE values of hematological parameters of various treatment groups

| Parameter | Day | Group I | Group II | Group III | Group IV |
|---|---|---|---|---|---|
| TEC ($x10^6/mm^3$) | 14 | $9.62±0.14^a$ | $5.60±0.43^b$ | $8.58±0.07^a$ | $6.70±0.07^c$ |
| | 28 | $8.54±0.62^a$ | $5.13±1.17^b$ | $8.13±0.57^a$ | $7.77±0.88^c$ |
| Hb Conc. (gm%) | 14 | $15.30±0.8^a$ | $9.20±0.60^b$ | $13.95±0.05^a$ | $11.25±0.25^c$ |
| | 28 | $13.20±0.6^a$ | $8.93±2.15^b$ | $13.67±0.9^a$ | $12.74±1.67^c$ |
| PCV (%) | 14 | $48.85±2.35^a$ | $34.65±2.85^b$ | $51.40±0.50^a$ | $46.20±0.50^c$ |
| | 28 | $49.35±2.15^a$ | $28.50±7.41^b$ | $50.37±1.12^a$ | $37.60±1.58^c$ |
| TLC ($x10^3/mm^3$) | 14 | $7.60±0.2^a$ | $6.35±0.45^b$ | $7.75±0.15^a$ | $6.90±0.40^c$ |
| | 28 | $7.40±0.2^a$ | $6.18±0.14^b$ | $7.90±0.21^a$ | $7.02±0.20^c$ |

**Table 2:** Mean±SE values of serum biochemical parameters of various treatment groups

| | Day | Group I | Group II | Group III | Group IV |
|---|---|---|---|---|---|
| AST (IU/L) | 14 | $71.37±4.09^a$ | $151.06±24.31^b$ | $75.06±23.28^a$ | $114.30±8.05^c$ |
| | 28 | $78.75±7.50^a$ | $187.30±12.11^b$ | $78.51±7.53^a$ | $134.42±3.43^c$ |
| ALT (IU/L) | 14 | $29.20±1.06^a$ | $57.49±5.28^b$ | $32.25±4.00^a$ | $40.40±2.25^C$ |
| | 28 | $27.22±6.27^a$ | $60.25±4.04^b$ | $29.44±1.42^a$ | $41.56±2.02^C$ |
| ALP (IU/L) | 14 | $92.54±3.74^a$ | $155.78±6.50^b$ | $97.53±7.74^a$ | $126.54±9.76^{bc}$ |
| | 28 | $102.43±3.85^a$ | $185.01±16.33^b$ | $95.42±3.76^a$ | $159.17±12.46^{bc}$ |
| Total Protein (g%) | 14 | 6.29±0.07 | 5.99±0.08 | 6.49±0.35 | 6.14±0.18 |
| | 28 | 6.88±0.39 | 6.70±0.10 | 7.34±0.37 | 6.53±0.21 |
| Creatinine (mg/dL) | 14 | $0.44±0.12^a$ | $0.82±0.06^b$ | $0.54±0.09^a$ | $0.62±0.14^a$ |
| | 28 | $0.74±0.08^a$ | $1.37±0.17^b$ | $0.62±0.05^a$ | $0.89±0.11^a$ |
| LDH (IU/L) | 14 | $911±75.0^a$ | $2786±145.0^b$ | $1002±78.0^a$ | $2334.5±88.5^c$ |
| | 28 | $1111.0±70.0^a$ | $2980.8±76.1^b$ | $1104±247.4^a$ | $2500.2±104.8^c$ |

## 4. Conclusions

Chemotherapy induced non-target organ toxicities has become a major constraint in clinical oncology. Although Imb was a proven drug in the management of various hematological and stromal cancers in both humans and animals, untoward cardiac, hepatic and renal toxicities were being reported. However, simultaneous usage of GSPs during Imb treatment may confer the organ protection through its proven mechanisms of cytoprotection and chemoprotection.

## 5. Acknowledgments

Authors are thankful to the Dean, Veterianry College – Bangalore, for providing facilities to carry out the present study.

## 6. References

1. Urruticoechea A, Alemany R, Balart J, Villanueva A, Viñals F, Capellá G. Recent Advances in Cancer Therapy: An Overview. Current Pharmaceutical Design. 2010; 16:3-10.
2. Ky B, Vejpongsa P, Yeh ETH, Force T, Moslehi JJ. Emerging paradigms in cardiomyopathies associated with cancer therapies. Circulation Research. 2013; 113:754-764.
3. Deininger M, Buchdunger E, Druker BJ. The development of imatinib as a therapeutic agent for chronic myeloid leukemia. Blood. 2005; 105:2640-2653.
4. Manley PW, Stiefl N, Cowan-Jacob SW, Kaufman S, Mestan J, Wartmann M *et al*. Structural resemblances and comparisons of the relative pharmacological properties of imatinib and nilotinib. Bioorganic and Medicinal Chemistry. 2010; 18:6977-6986.
5. Bonkobara M. Dysregulation of tyrosine kinases and use of imatinib in small animal practice. The Veterinary Journal. 2015; 205:180-188.
6. Kerkela R, Grazette L, Yacobi R, Iliescu C, Patten R, Beahm C *et al*. Cardiotoxicity of the cancer therapeutic agent imatinib mesylate. Nature Medicine. 2006; 12:908-916.
7. Al-Rasheed NM, El-Masry1 TA, Tousson E, Hassan HM, Al-Ghadeer A. Hepatic protective effect of grape seed proanthocyanidin extract against Gleevec-induced apoptosis, liver Injury and Ki67 alterations in rats. Brazilian Journal of Pharmaceutical Sciences. 2018; 54(2):e17391.
8. Abbas A, Mirza MM, Ganti AK, Tendulkar K. Renal toxicities of targeted therapies. Target Oncol. 2015; 10(4):487-499.
9. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavanoids and phenolic acid. Free Radical Biology & Medicine. 1996; 20:933-956.
10. Bagchi D, Bagchi M, Stohs SJ, Das DK, Ray SD, Kuszynski CA *et al*. Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. Toxicology. 2000; 148:187-197.
11. Abirami M, Kanagavalli U. Cardioprotective effect of grapeseed proanthocyanidin on doxorubicin induced myocardial injury in rats. International journal of Pharmacy & Life Sciences. 2013; 4(1):2288-2293.
12. Bagchi D, Swaroop A, Preuss HG, Bagchi M. Free radical scavenging, antioxidant and cancer chemoprevention by grape seed proanthocyanidin: An overview. Mutation Research. 2014; 768:69-73.
13. Xu Z, Cang S, Yang T, Liu D. Cardiotoxicity of tyrosine kinase inhibitors in chronic myelogenous leukemia therapy. Hematology Reviews. 2009; 1:e4.
14. Herman EH, Knapton A, Rosen E, Thompson K, Rosenzweig B, Estis J *et al*. A multifaceted evaluation of imatinib-induced cardiotoxicity in the rat. Toxicologic

Pathology. 2011; 39:1091-1106.
15. Saad SY, Alkharfy KM, Arafah MM. Cardiotoxic effects of arsenic trioxide/imatinib mesilate combination in rats. Journal of Pharmacy and Pharmacology. 2006; 58:567-573.
16. Hassan NAM, Yousef MM. Study of imatinib cardiotoxicity in adult male rabbits. IOSR Journal of Environmental Science, Toxicology and Food Technology. 2006; 6(5):14-26.
17. Haq MI, Nixon J, Stanley AJ. Case report on Imatinib and liver toxicity. BMJ Case Rep. 2018; 11(1-4):e226740. doi:10.1136/bcr-2018226740
18. Deininger MWN, O'Brien SG, Ford JM, Druker BJ. Practical management of patients with chronic myeloid leukemia receiving imatinib. Journal of Clinical Oncology. 2003; 21:1637-1647.
19. Gafter-Gvili A, Ram R, Gafter U, Shpilberg O, Raanani P. Renal failure associated with tyrosine kinase inhibitors-Case report and review of the literature. Leukemia Research. 2010; 34:123-127.
20. Buchdunger E, Cioffi CL, Law N, Stover D, Ohno-Jones S, Druker BJ *et al*. Abl protein-tyrosine kinase inhibitor STI571 inhibits *in vitro* signal transduction mediated by c-kit and platelet-derived growth factor receptors. The Journal of Pharmacology and Experimental Therapeutics. 2000; 295(1):139-145.
21. Floege J, Eitner F, Alpers CE. A new look at platelet-derived growth factor in renal disease. Journal of the American Society of Nephrology. 2008; 19(1):12-23.
22. Vidal-Petiot E, Rea D, Serrano F, Stehlé T, Gardin C, Rousselot P *et al*. Imatinib increases serum creatinine by inhibiting its tubular secretion in a reversible fashion in chronic myeloid leukemia. Clinical Lymphoma, Myeloma & Leukemia. 2016; 16(3):169-174.
23. Nazima B, Manoharan V, Miltonprabu S. Grape seed proanthocyanidins ameliorates cadmium-induced renal injury and oxidative stress in experimental rats through the up-regulation of nuclear related factor 2 and antioxidant responsive elements. Biochemistry and Cell Biology. 2015; 93(3):210-226.