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## Histopathological studies on imatinib mesylate induced toxicity in wistar rats and its amelioration with grape seed proanthocyanidins

**I Hemanth, HD Narayanaswamy, ML Satyanarayana, Suguna Rao, KV Jamuna and Prakash Nadoor**

### Abstract

Non-target organ toxicities associated with chemotherapy has been a major setback in the clinical usage of many efficient anticancer drugs. In similar terms, imatinib mesylate, an important member of Tyrosine kinase inhibitor class of drugs has also been associated with vital organ toxicity. Grape seed proanthocyanidins were popular chemoprotective agents against various drug/chemical induced toxicities with proven anti-oxidant mechanisms. In this context, the present study was undertaken to investigate the ameliorative efficacy of GSPs against imatinib induced toxicity in Wistar male rats through histopathological evaluation. The results indicated significantly pronounced lesions of toxicity among drug positive control animals, especially involving the heart, liver and kidneys. GSPs concurrently administered group, however manifested similar lesions of milder intensity, suggesting the ameliorative and chemoprotective efficiency of GSPs against imatinib mesylate induced non-target toxicity.

**Keywords:** Grape seed proanthocyanidins, heart, histopathology, imatinib mesylate, kidney, liver, rats

### 1. Introduction

Chemotherapy has become an important treatment modality in cancer treatment among human and canine patients. However, the associated toxicity during the treatment has been an issue of major concern. To counteract these un-warranted side effects, targeted therapies that targets key molecules of tumor biology are the recent advancements with an additional objective of minimizing the non-target toxicities<sup>[1]</sup>.

Tyrosine kinase inhibitors (TKIs) are the novel members of Targeted therapy. They function by inhibiting the receptors or signaling molecules of aberrantly functioning tyrosine kinase pathways, which are the key signaling pathways driving the tumor progression. Imatinib mesylate is one among the first members of TKI family of targeted chemotherapeutic drugs that was initially introduced for the treatment of chronic myeloid leukemia in humans, wherein BCR-ABL fusion protein of Philadelphia chromosome constitutively exerts the tyrosine kinase activity driving the myeloid cells towards leukemia<sup>[2]</sup>. Later on therapeutic efficacy of imatinib was evident even in the cancers of dog and cat *viz.* mast cell tumors, gastro-intestinal stromal tumors, melanomas, leukaemia/lymphoma, mammary gland carcinomas, thyroid carcinoma, haemangiosarcoma *e.t.c.*<sup>[3]</sup>. However, the unanticipated organ toxicities especially of heart is of prime concern during imatinib therapy with the pathogenic mechanisms playing at various sub-cellular levels<sup>[4]</sup>. In addition, toxicities pertaining to other vital organs like liver and kidneys has also been reported, thus hindering its clinical use<sup>[5, 6]</sup>.

Grape seed proanthocyanidins (GSPs) are the polyphenolic phytochemicals with well documented spectrum of biological, pharmacological, therapeutic and chemoprotective properties against free radicals and oxidative stress<sup>[7, 8]</sup>. Their beneficial properties have also been demonstrated in significant *in vivo* protection against structurally diverse drug and chemical-induced hepatotoxicity, cardiotoxicity, neurotoxicity, nephrotoxicity and spleenotoxicity, in various experimental studies<sup>[9, 10]</sup>. With this background, the present study was undertaken to study the possible amelioration of imatinib induced non-target toxicity by GSPs in Wistar rats.

## 2. Material and Methods

### 2.1 Drug and GSP extract procurement

Active pharmaceutical ingredient of Imatinib was procured from M/S. Vibgyor Drugs Pvt. Ltd., Hyderabad 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) upon Institutional Animal Ethics Committee (IAEC) approval. 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 Imatinib 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 Imatinib @ 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<sup>th</sup> and 28<sup>th</sup> Days under chloroform over-dose euthanasia.

### 2.4 Histopathology

Representative tissue samples from all the organs were collected during each sacrifice upon gross examination and fixed in 10% neutral buffered formalin. Tissue samples were subjected for routine histological processing with paraffin embedding, microtomy sectioning and routine H&E staining [11].

## 3. Results

All the visceral organs from Groups I and III appeared normal with regular histological features. While the lesions were most conspicuous in heart, liver and kidneys among Group II animals, they were seen in reduced magnitude among Group IV animals.

**3.1 Heart:** Group II animals revealed a range of degenerative changes involving the cardiac myocytes on Day 14 *viz.* eosinophilic, granular and vacuolar sarcoplasm (Fig. 1) along with inter-fibrillar edema (Fig. 2). Diffuse areas of hemorrhages were also noticed between muscle fibers (Fig. 3). Necrotic cells with karyolytic changes and apoptotic cells with condensed and pyknotic nucleus were also observed at few places (Fig. 4). By Day 28, degenerative, necrotic and

apoptotic changes were more severe with extensive sarcoplasmic vacuolization and hyalinization of individual cells. In addition, mononuclear infiltration was observed adjacent to fragmented and necrosed myocytes along with few fibroblasts (Fig. 5).

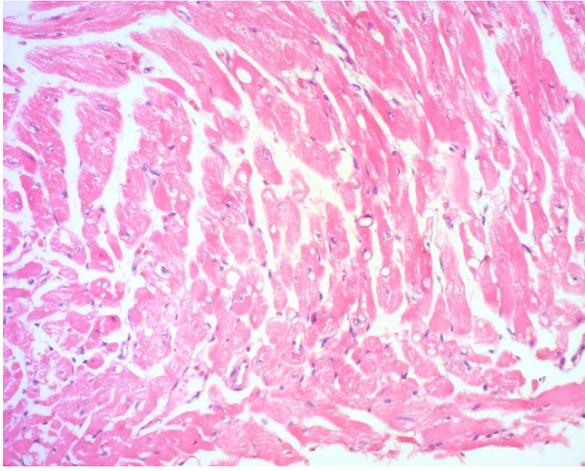
In Group IV animals, similar lesions were noticed but in reduced magnitude. Mild to moderate degenerative and vascular changes and focal areas of necrotic changes were observed. The lesions were found to be further declining in the intensity from Day 14 to Day 28 of the study.

**3.2 Liver:** Group II animals on Day 14 revealed mild congestion, swollen hepatocytes with granular cytoplasm. Fatty change with large clear vacuoles in the cytoplasm were noticed. By Day 28, there was an increase in the severity of lesions. Cytoplasmic vacuoles were numerous and small, with or without displacing the nucleus, suggesting glycogen infiltration (Fig. 6). The identity of glycogen was confirmed by Periodic Acid Schiff's method (Fig. 7). A number of pre-apoptotic cells which were angular and eosinophilic with condensed nucleus were also observed. Another important observation was presence of single cell necrosis of hepatocytes giving a starry sky appearance. Such cells were distinct from normal hepatocytes and appeared either as spaces with hazy cytoplasm and disintegrating nucleus or as empty spaces (Fig. 8). In addition, multi-focal areas of necrosis were observed with infiltration of inflammatory cells (Fig. 9).

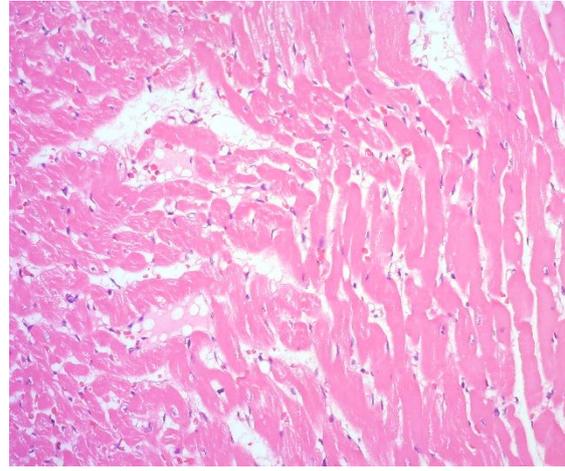
Group IV animals on Day 14 revealed moderate lesions such as cell swelling and granularity of the cytoplasm. Occasional cells with fatty change and glycogen infiltration were evident. Scattered areas of single cell necrosis were also noticed. By Day 28, there was a total decline in the occurrence of the lesions with only occasional cells manifesting the fatty change. There was an improvement in the architecture of liver parenchyma.

**3.3 Kidneys:** Group II animals on Day 14 revealed degenerative changes involving the tubular epithelial cells. These were characterized by cell swelling and vacuolar cytoplasm, with obvious reduction in tubular luminal space. Apoptosis and necrosis of lining epithelial cells were evident in some tubules. Congestion of interstitial vessels especially in the medullary region was observed. On Day 28, tubular epithelial cells showed severe cytoplasmic vacuolations compressing the nucleus to baso-lateral margins of the cells (Fig. 10). Sometimes, disruption of the cell membranes was also evident. Some of the proximal and distal convoluted tubules showed necrosis of lining epithelial cells (Fig. 11), along with desquamation, leaving the bare basement membranes. Apoptosis was also a characteristic finding in certain tubules, characterized by dark and condensed nucleus (Fig. 12). Glomeruli were found to be less affected except for mild congestion.

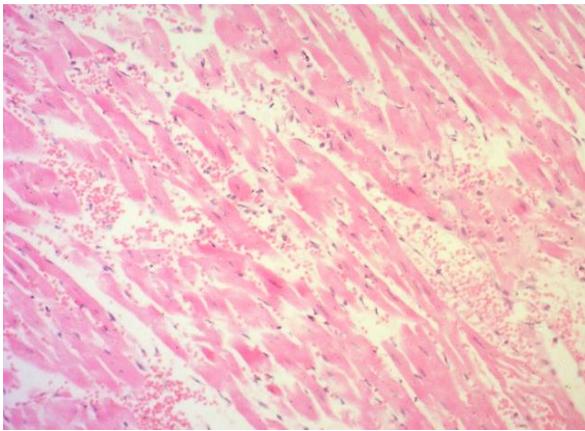
In Group IV, similar degenerative, necrotic and apoptotic lesions were observed, however of lesser severity. On Day 14, moderate vacuolar degeneration of tubular epithelial cells with no evidence of apoptosis was observed. On Day 28, near normal architecture was evident in the kidneys with very few lesions like congestion.



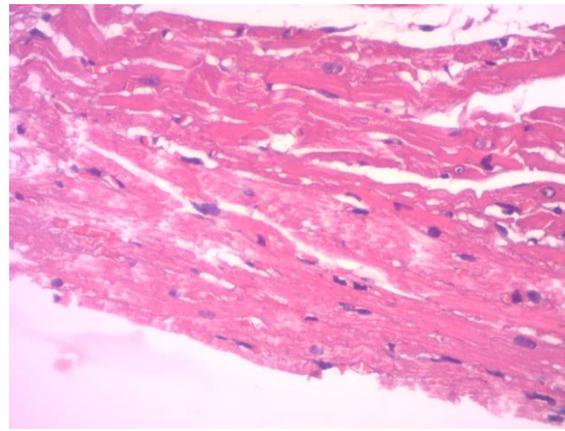
**Fig 1:** Group II-Heart-Sarcoplasmic vacuolations along with degenerative changes in cardiomyocytes. H&E X200



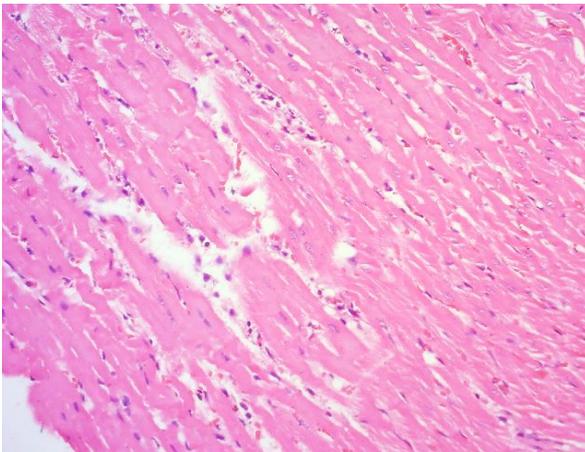
**Fig 2:** Group II-Heart- Interstitial oedema and widening of interfibrillar spaces within the cardiomyocytes. H&E X200



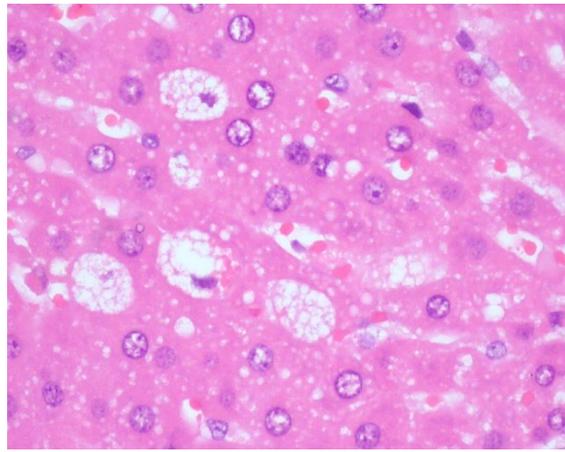
**Fig 3:** Group II-Heart- Myocytolysis and presence of RBCs, fibrocytes and mono-nuclear cells within the interstitium. H&E X100



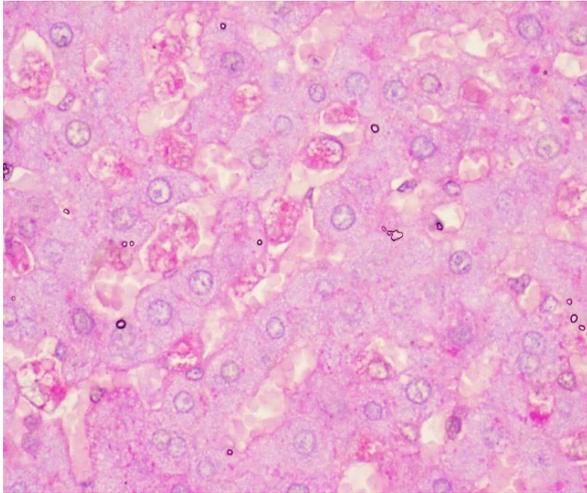
**Fig 4:** Group II-Heart- Severe injury with degeneration and necrosis of cardiomyocyte fibres. H&E X400



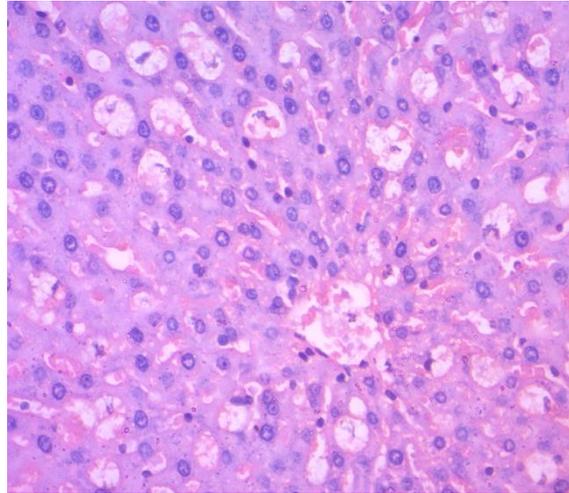
**Fig 5:** Group II-Heart- Mononuclear cells infiltrations amidst the damaged cardiomyocytes. H&E X100



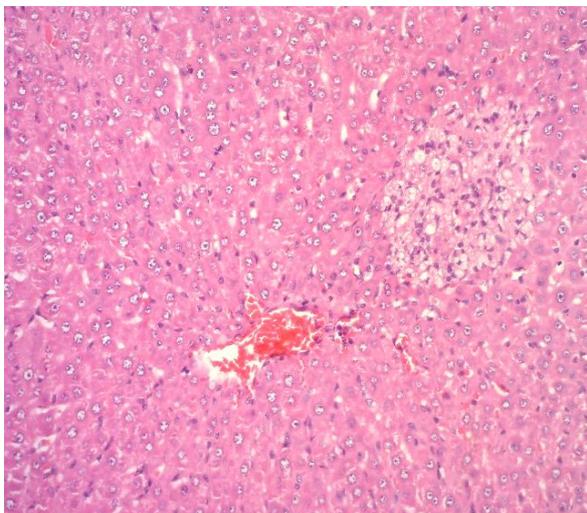
**Fig 6:** Group II-Liver- Hepatocytes revealing vacuolar cytoplasm suggestive of glycogen infiltration. H&E X400



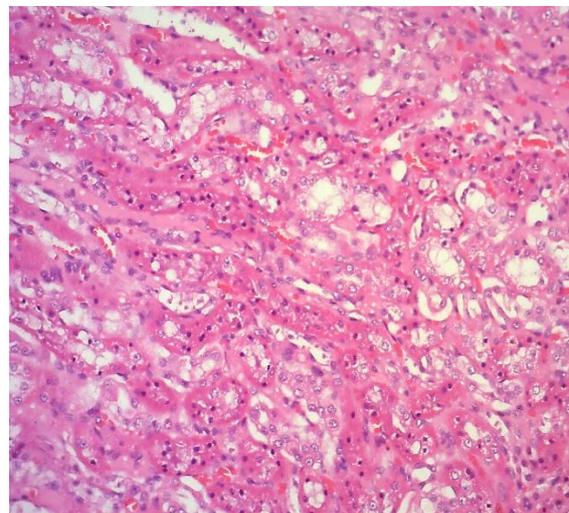
**Fig 7:** Group II-Liver- Hepatocytes revealing pinkish cytoplasmic granules of glycogen infiltration. PAS X400



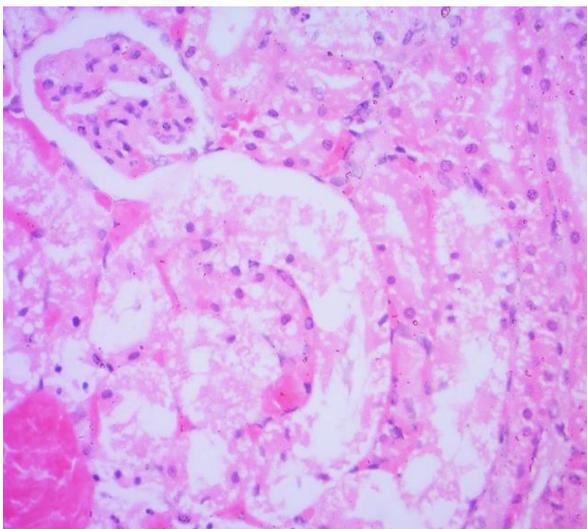
**Fig 8:** Group II-Liver- Hepatocytes revealing various stages of single cell necrosis. H&E X400



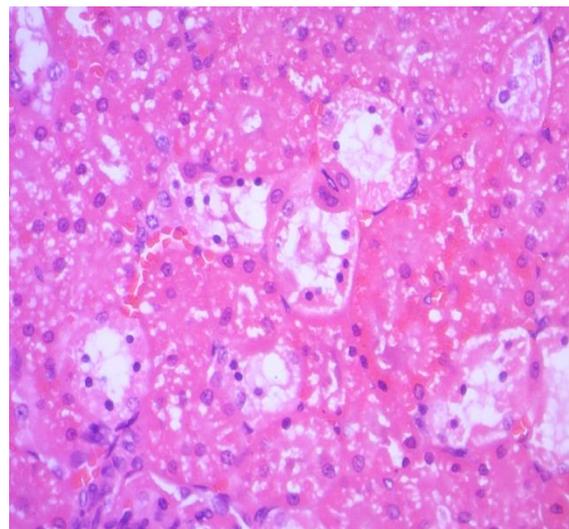
**Fig 9:** Group II-Liver- Focal areas of necrosis with karyolytic changes and infiltration of inflammatory cells. H&E X200



**Fig 10:** Group II-Kidney- Severe cytoplasmic vacuolations of tubular epithelial cells. H&E X200



**Fig 11:** Group II-Kidney- Necrosis and desquamation of epithelial cells lining the proximal and distal convoluted tubules. H&E X200



**Fig 12:** Group II-Kidney- Multiple apoptotic cells with pyknotic nucleus among the tubular epithelial cells. H&E X400

#### 4. Discussion

Molecular targeted anticancer agents, although assumed to lack the cytotoxic side-effects of traditional chemotherapies, they can still introduce new challenges to optimize drug therapy [12]. With regard to imatinib mesylate, in spite of being a successful choice as targeted therapy against kinases dys-functioning neoplasms, it has been reported to cause certain untoward side-effects *viz.* unanticipated cardiotoxicity in human patients [13, 14] and toxic effects involving the bone marrow, hepatic, renal and gastrointestinal systems in dogs and cats, at clinically relevant doses [3]. Although not completely elucidated, the proposed mechanisms of imatinib induced toxicity include 'On-target' and 'Off-target' mechanisms [15].

The primary mechanisms by which imatinib induces cardiotoxicity are induction of oxidative stress [16, 17], initiation of endoplasmic reticulum (ER) stress [13, 15, 18] and through mitochondrial dysfunction [19]. Histopathological lesions in heart revealed the lesions indicative of ongoing cardiac injury and were all suggestive of drug induced cardiotoxicity, as described in National Toxicology Program (NTP) studies [20]. Similar lesions were reported in various other earlier studies on imatinib and doxorubicin induced cardiotoxicity in mice, rats and humans [21, 22].

Liver being the main site for bioactivation and detoxification of various xenobiotics including different classes of drugs, it is the most commonly affected organ with drug toxicity. Small molecule TKIs including imatinib were assumed to be associated with a relatively high incidence of drug induced liver injury as a group, among human patients [23]. Lesions pertaining to hepatic toxicity in the present study were in agreement with earlier reports in human [24] and veterinary literature [3]. The postulated mechanisms of toxicity include: metabolic activation (bio activation) and formation of toxic reactive intermediates [25] and mitochondrial disruption and cell damage [26].

Imatinib mesylate induced renal toxicity was well exemplified among humans and animal patients [3, 27], which was evident even in the present study. The principal mechanisms of renal toxicity were mediated by the inherent property of the drug to inhibit PDGF-PDGFR signaling pathways, which are crucial for the tubulogenesis activity and restoration of renal functioning upon acute tubular injury of any etiology. Thus, off-target inhibition of this critical pathway may interfere with repair of tubular damage that arises at least due to drug induced oxidative damage [28]. Thus, the histopathological observations in kidneys were suggestive of acute tubular injury and were in agreement with earlier reports [5, 27, 29].

GSPs conferred vital organs protection observed in the current study could be due to their over-all health preservation properties that counteracts various drug induced toxicities [30]. Since, oxidative stress at cellular level and subsequent cell injury has been postulated as one of the diverse mechanisms of imatinib induced cellular damage, the potential anti-oxidant properties of GSPs might have counteracted and protected the drug induced cardiac, hepatic and renal injury [31, 32]. However, identifying and establishing the molecular mechanisms underlying such properties of GSPs would be helpful in understanding their actual mechanisms of toxic amelioration.

#### 5. Conclusions

Non-target organ toxicities of targeted therapies including that of imatinib mesylate has been a major hindrance in their

clinical usage as chemotherapy. However, the extent of such toxicities can be ameliorated, although not completely, by the concurrent usage of certain natural chemoprotective agents like GSPs which can confer protection against drug induced toxicity through their potent anti-oxidant defense mechanisms.

#### 6. Acknowledgments

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