The present studies were intended towards finding a suitable plant based natural product which can ameliorate the hematotoxicity encountered as adverse drug reactions (ADRs) in various therapeutic regimens. *Dillenia indica* (DI) is used in folklore and traditional medicine as general health enhancer. The aim of the study was to evaluate the hematoprotective effects of natural botanical, *Dillenia indica* (DI) ethanolic extract (DIEE), employing a Phenylhydrazine (Phz)-induced hematotoxicity model of Sprague Dawley (SD) rats. DIEE exhibited hematoprotective effects *In vitro* and *In vivo*. Solvent guided Fractionation studies revealed the presence of high amounts triterpenoids in DIEE. DIEE showed radical scavenging properties in DPPH radical scavenging assay. Mechanism based studies unveiled the positive effects of DIEE on antioxidant enzymes Superoxide dismutases 2 and 3 (SOD’s) and Nuclear erythroid 2 p45-related factor 1 (Nfer-1) and Nuclear erythroid 2 p45-related factor 2 (Nfer-2) employing quantitative real time PCR. In toxicity studies in SD rats, DIEE imparted no toxicity. The results are of integral significance as, the study establishes the folklore and traditional medicine claims about the peremptory actions of DI.

**Keywords:** Hematoprotective, *Dillenia indica* L., Anti-oxidants, Free-radical, Anti-oxidant intrinsic defence

**IPC Int. Cl.** A61K 36/00, C09K 15/00, C07B 61/00

Various drugs pose risk to the biological system in terms of adverse drug reactions (ADR’s) and blood being an important tissue gets most exposed to such agents which are targeted to suppression of cell proliferation. Any deformity or change in Reactive Oxygen Species (ROS) threshold leads to loss of Red Blood Cells (RBCs) structure leading to loss of function. RBCs may undergo hemolysis, protein cross-linking and changes in membrane fragility and function leading to changes in the conformation of the membrane proteins. Hemoglobin molecule has a tendency to undergo auto-oxidation and gives rise to endogenously generated H$_2$O$_2$ which becomes detrimental for the erythrocytes.

Optimum levels of Reactive Oxygen Species (ROS) are important for the normal functioning of the biological system. Ideally, there should be a very fine balance between the ROS, pro-oxidants and anti-oxidants that shall mediate the proper functioning of the living system. Just when this equilibrium is disrupted, system comes under oxidative stress which then leads to oxidative damage. This enhanced stress in the system leads to easy oxidation of various biomolecules which are lipids, carbohydrates and proteins, which are integral for life. Nonetheless, complete elimination of ROS from system is not recommended at all as it would be detrimental to the system.

The innate defences provided to maintain cellular integrity within normal functioning cells to combat oxidative injury include enzyme systems referred as anti-oxidant enzyme systems, viz. SOD’s, Catalase, Nfer’s, Glutathione peroxidases (GPx) and peroxiredoxines. Considering the extent of oxidative stress burden particularly in response to specific treatment regimes, faced by blood, it is most prudent to come up with specific hemato-protective agents that can counter such adversities. Amongst multitude of sources for medicinal agents, therapies originating out of natural sources have immense promise because of their long known use in traditional practices and because of comparatively low adverse effects. The studies were performed towards evaluating a natural Botanical *Dillenia indica* (DI) for its effects on...
hematotoxicity induced by Phenylhydrazine in a Sprague Dawley rat model. *Dillenia indica* belongs to family *Dilleniaceae* which is characterized by significant morphological variations in the genera belonging to this family. Amongst plants belonging to genus *Dillenia*, *D. pentagyna*, *D. suffruticosa* and *D. indica* are of prime importance because of their uses in traditional medicine.

**Material and methods**

**Chemicals and kits**

DMSO (Dimethyl Sulphoxide), 0.9% Saline, Methanol, DPPH (2,2-diphenyl-1-picrylhydrazyl), Triton X-100, Phenylhydrazine (Phz), Concentrated H$_2$SO$_4$, Chloroform, acetic anhydride and RNAzol were obtained from sigma Aldrich, India. EDTA (EthylenediamineTetraacetic Acid) coated vials obtained from Polymedicure limited, India. SYBR Green and cDNA synthesis kit were obtained from Thermo Scientific. Primers were obtained from Integrated DNA Technologies. For qPCR studies, beta actin was used as control.

**Ameliorative effects of DIEE on CBC in rats**

**Collection and preparation of DIEE**

The research material for present studies, i.e., *Dillenia indica* L. (Leaves) were procured from South Andaman region, Andaman and Nicobar Islands vide field Voucher No. 18731.

**Ameliorative effects of DIEE**

DIEE was administered at dose of 100, 200 and 400 mg/kg body weight to the test animals as per their respective treatment groups.

**Animals and animal care**

Female Sprague-Dawley albino rats weighing between 120-150 gm were used in the study. Institutional Animal Ethics Committee (IAEC) approval (No. IAEC/2013/70) was obtained.

**Study design and grouping of animals:**

Sixteen female rats were randomly assigned to 4 treatment groups, each group consisting of 4 female animals and one group comprising of an equal number of animals served as control.

**Induction of hematotoxicity**

Phenylhydrazine is a known hematotoxicant. It was administered orally to all groups (except control) at the dose of 8 mg/kg body weight daily.

**Extraction and fractionation of DIEE**

DI extract in ethanol was obtained by soaking the dried plant material for 72 hrs. The obtained solvent was completely evaporated under reduced pressure below 50 °C up to complete removal of traces of solvent to obtain a thick, viscous extract termed as DIEE. DIEE was used in the study and further partitioned in 10% aqueous methanol (MF), n-hexane (NHF).

**Phytochemical screening of DIEE fraction**

Libermann-Buchard and Salkowski test was performed as described by Yadav *et al*.

**Effect on erythrocytes**

Method followed was from Sivonova *et al.* with slight modifications.

**RBC isolation**

Blood samples were drawn from healthy rats in EDTA coated tubes and centrifuged at 2,000 rpm for 10 min. The obtained packed RBCs were washed thrice in 0.9% saline and subjected to effect of DIEE.

**Preparation of plant solution and dilutions**

Stock solutions (10mg/ml) of DIEE fractions, were prepared in DMSO. These were then diluted upto 3 folds.

**Measurement of hemolysis**

Positive control was 1% triton X-100; the detergent which is known to induce hemolysis. Whereas saline served as the negative control at pH 7 maintains the RBC structure.

**Anti-oxidant activity of DIEE**

The procedure described by Grace *et al.* 2012 was followed.

**Effect of DIEE on anti-oxidant enzyme system**

**Study design and grouping of animals**

Twelve rats were randomly assigned to 2 treatment groups, one control another one as toxin control; each group consisting of 4 female animals. One group comprising of an equal number of animals served as control.

**RNA isolation and cDNA preparation**

RNA isolation was performed by RNAzol method as prescribed by manufacturer’s protocol. Total RNA was isolated from blood. This quantified RNA was the converted into cDNA using Revert aid First strand cDNA synthesis kit from Thermo Scientific as prescribed by manufacturer’s protocol.
Quantitative real-time PCR
Fold change of rat Nfer-1, Nfer-2, SOD-1 and SOD-2 were measured by single step PCR (RT-PCR).

Sub-chronic toxicity of DIEE
Study design and grouping of animals
Twenty five rats were randomly assigned to 3 treatment groups, each group consisting of 5 female animals.

Dosing with DIEE
DIEE was dosed to the treatment experimental group at 100, 200 and 400 mg/kg body weight daily for 54 days. The DIEE was mixed in fresh water daily just prior to dosing.

Hematological analyses
Initial and final CBC’s were recorded and analysed for any chronic toxicity effects of DI on the experimental animals.

Statistics
The data generated was subjected to t-test for calculating the value of significance ($p$ value) using graph pad prism. Data are expressed as Mean±S.D. A value of $P \leq 0.05$ was used as a criterion for significance.

Results
Ameliorative effects of DIEE on CBC in rats

Induction of hematotoxicity
Dosing of Phenylhydrazine induced significant hematotoxicity in rats. In initial hematology report, all the groups exhibited normal values of blood parameters. After 7 days of dosing with Phz, there was a significant reduction in the counts of various blood parameters; Hgb, RBC, Hct and MCHC exhibiting 50%, 60%, 36% and 31% reduction, respectively. On the other hand, the counts of MCV, MCH, TLC and Platelets exhibited a significant 114%, 78%, 73% and 56% increase, respectively. The counts of subjects from control group were unaltered.

Attenuating effect of DIEE
The treatment of hemato-compromised rats with DIEE for 7 days was observed to induce recovery in altered blood parameters. The restoration of Hgb, RBC, Hct, MCHC was 94%, 150%, 50%, 43% (increased), respectively when compared to hemato-compromised condition. The blood parameters which were increased form their normal values in the hemato-compromised condition were also restored to normal with decrease of about 53%, 46%, 43%, 33% in MCV, MCH, TLC and Platelets respectively as described in Table 1.

Fractionation of DIEE
Ethanol was the main solvent that was used in the experiments. The fractionation patterns of DI depicted that its activity falls in the non-polar ranges.

Phytochemical screening of DIEE fraction
n-hexane fraction was found most active in the studies. Phytochemical screening of n-hexane fraction revealed the presence of triterpenoids. In Libermann-Buchard test occurrence of red color predicted the presence of triterpenoids. Concurrent results were obtained in Salkowski test, where formation of a yellow colour layer depicted the presence of triterpenoids in n-hexane fraction.

Effect on erythrocytes
Samples obtained EF (Ethanolic fraction), MF (Methanolic fraction) and NHF (n-Hexane fraction) were tested for their hematoprotective activities on isolated erythrocytes. Amongst EF, MF and NHF, NHF shows the maximum activity when compared with Triton X-100, which served as the positive control. At 10mg/ml dilution, the per cent change

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hgb (gm/dl)</th>
<th>RBC (X10³µL)</th>
<th>Hct (%)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (gm/dL)</th>
<th>TLC (X10³µL)</th>
<th>Platelet (X10³µL)</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>10.7±0.38</td>
<td>6.3±1.02</td>
<td>35.5±3.5</td>
<td>60.2±5.</td>
<td>3.13±1.6</td>
<td>31.38±2.4</td>
<td>12.5±0.6</td>
<td>380±06.17</td>
</tr>
<tr>
<td>II(a)</td>
<td>5.2±0.29***</td>
<td>2.5±1.18***</td>
<td>24.1±3.3</td>
<td>122.2±26.5***</td>
<td>22.6±1.8***</td>
<td>21.78±1.16***</td>
<td>23.9±2.5***</td>
<td>665±161.32***</td>
</tr>
<tr>
<td>II(b)</td>
<td>10.3±1.44***</td>
<td>6.19±0.56***</td>
<td>37.45±0.9***</td>
<td>58.6±1.9***</td>
<td>12.1±1.24***</td>
<td>31.23±5.03***</td>
<td>13.9±2.56***</td>
<td>428±53.11***</td>
</tr>
<tr>
<td>III(a)</td>
<td>5.4±0.87***</td>
<td>2.43±0.62***</td>
<td>23±1.9***</td>
<td>120.2±11.9***</td>
<td>22.9±1.5***</td>
<td>21.9±1.02***</td>
<td>24.48±1.0***</td>
<td>614±177.2***</td>
</tr>
<tr>
<td>III(b)</td>
<td>10.4±0.68***</td>
<td>6.29±0.84***</td>
<td>37.18±3.1***</td>
<td>58.8±4.86***</td>
<td>12±0.83***</td>
<td>30.98±1.19***</td>
<td>13.4±0.94***</td>
<td>437.7±68.5***</td>
</tr>
<tr>
<td>IV(a)</td>
<td>5.3±0.4***</td>
<td>2.48±0.81***</td>
<td>24.1±1.5***</td>
<td>140.5±18***</td>
<td>22±1.08***</td>
<td>21.5±1.1***</td>
<td>23.3±1.7***</td>
<td>652.7±3.6***</td>
</tr>
<tr>
<td>IV(b)</td>
<td>10.2±0.48***</td>
<td>6.1±1.22***</td>
<td>37.15±3.6***</td>
<td>60.05±5.7***</td>
<td>12.2±0.7***</td>
<td>31.4±2.8***</td>
<td>13.1±1.6***</td>
<td>405.7±63.59***</td>
</tr>
</tbody>
</table>
from Triton X-100 was 69.06% 92.79% and 95.19% for EF, MF and NHF, respectively. At next dilution of 1mg/ml the values were 84.38%, 93.09% and 94.59% for the respective fractions. At a dilution of 0.1mg/ml, the percentage change in respect of Triton X-100 was 86.78%, 93.99% and 94.29%. The results suggest that amongst all the fractions obtained, the n-hexane fraction at each dilution shows maximum hematoprotective activity.

**Anti-oxidant activity of DIEE**

At varying concentrations of 20, 40, 60, 80, 100, 200 and 400 µg/ml of DIEE showed radical scavenging of DPPH as depicted in Fig. 1.

**Effect of DIEE on anti-oxidant enzyme system**

**Hematological analyses**

At the dose of 400 mg/kg body weight, DI completely ameliorated Phz induced hematoxicity. All the blood parameters that were altered under Phz treatment were restored to normal (Table 2).

Hgb tends to be low after a 7 day treatment of Phz, which was recovered with 7 day treatment of DIEE at 400 mg/kg body weight. Control Group 1 showed normal ranges at both hematological examinations (day 7 and day 14), whereas, toxin control (Group 2) remained with the lowered values (% increase = 67.21) from previous hematological examination. Whereas, group receiving DIEE treatment at the dose of 400 mg/kg Body weight (treated group) has showed complete recovery with per cent increase of 129.24%.

For RBC, Control (Group 1) exhibited normal range at both hematological examinations (day 7 and day 14). Toxin control (Group 2) remained with the lowered values whereas, group receiving DIEE treatment showed complete recovery of 201%.

The levels of Hct, which were significantly reduced after 7 days treatment of Phz, were recovered with 7 day treatment of DIEE. The recovery in toxin control was 18% whereas in the treatment group it was 47%.

With 7 days dosing, MCV increased significantly in both treated and toxin control groups. While, after treatment with DIEE, treated group attained normal value (% decrease = 45%) while toxin control remained at higher values thereafter. Values of MCH with 7 days dosing of Phz, were recovered with 7 day treatment of DIEE. The recovery in toxin control was 7% whereas in the treatment group it was found to be 23%. MCHC and TLC were decreased by Phz treatment and were restored after treatment with DIEE. The recovery in toxin control was 29% and 9%; whereas, in treatment group it was 55% and 34% for MCHC and TLC, respectively. For Platelets, toxin control and treatment groups recovery observed was 8% and 12%.

**Effect on SODs**

In order to explore the mechanistic aspects of the effect of DI, we quantified the expression of various anti-oxidant genes by quantitative Real Time PCR studies. The studies showed that mRNA expression of SOD 1 and SOD 2 were higher in the treatment groups receiving DIEE (T.2) compared to the toxin control (T.1) (Figs. 2 a & b).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hgb (gm/dl)</th>
<th>RBC (X10^6µL)</th>
<th>Hct (%)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (gm/dL)</th>
<th>TLC (X10^3µL)</th>
<th>Platelet (X10^3µL)</th>
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</thead>
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<tr>
<td>I</td>
<td>13.1±1.0</td>
<td>7.5±1.0</td>
<td>40.4</td>
<td>115.5±3.</td>
<td>19.0±2</td>
<td>21.8±2.5</td>
<td>14.8±5.4</td>
<td>325.4±113.2</td>
</tr>
<tr>
<td>II(a)</td>
<td>5.4±0.7***</td>
<td>2.1±0.3***</td>
<td>26.7±2.8**</td>
<td>122.1±7.9***</td>
<td>25.0±1.4**</td>
<td>20.3±1.03***</td>
<td>28.9±4.7***</td>
<td>741.5±88.58***</td>
</tr>
<tr>
<td>II(b)</td>
<td>9.0±1.7***</td>
<td>4.0±0.5***</td>
<td>21.7±4.6**</td>
<td>88.5±4.0***</td>
<td>23.2±1.54**</td>
<td>26.2±1.0***</td>
<td>29.1±5.1***</td>
<td>756.4±128.7***</td>
</tr>
<tr>
<td>III(a)</td>
<td>5.3±0.55***</td>
<td>2.0±0.1***</td>
<td>26.2±4.0**</td>
<td>112.6±11.2**</td>
<td>26.09±2.4**</td>
<td>20.2±1.1**</td>
<td>27.9±5.0**</td>
<td>803.5±122.3***</td>
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<tr>
<td>III(b)</td>
<td>12.15±1.7³</td>
<td>6.1±0.7³</td>
<td>38.6±5.1³</td>
<td>60.85±4.8³</td>
<td>18.26±0.72³</td>
<td>31.4±1.3³</td>
<td>18.82±2.1³</td>
<td>502.0±140.4³³</td>
</tr>
</tbody>
</table>

Table 2- Quantitative analyses of various hematological endpoints at different time intervals. II(a) and III(a) Complete Blood Count profile after 7 days dosing of Phenylhydrazine. III(b) Complete Blood Count profile after 7 days dosing of DIEE. Group I is Control, Group II Toxin control and Group III is DIEE treated. [*p<0.05; **p<0.005; ***p<0.001; δ=ns; compared with Control*]
**Effect on Nfers**

The quantification of Nfer 1 and 2 gene expression revealed that the m-RNA expression levels of DIEE treated groups (T.2) were significantly higher than the Phz treated groups (T.1) (Figs 3 a & b).

**Sub-chronic toxicity of DIEE**

In 54 day toxicity study of DIEE no toxicity was observed in initial and final hematological reports (Table 3).

**Discussion**

Phenyldihydrazine (Phz) induces hematotoxicity via free radical generation which leads to the deleterious effects to the biological system⁷. Phz induces damage to erythrocytes by damaging the RBC membrane followed by the release of iron which results in the complete loss of structure and function for RBC¹². This resulted oxidative stress is pernicious to the system which leads to severe adverse effects. Resultant disturbance of pro and anti-oxidants possesses serious threat to the survival of the cellular machinery¹¹. Amongst other cells, erythrocytes and other blood cells show a higher susceptibility towards oxidative stress². *Dillenia* species have been used by healers of folklore medicine and the plant finds its integral importance in traditional medicine¹⁴. DI fruits are eaten raw for general health enhancement¹⁵. Considering the peremptory actions of strong anti-oxidants in blood ailments *Dillenia indica* ethanolic extract (DIEE) was tested on Phenyldihydrazine treated rats. The results obtained suggest that the blood parameters which were compromised after the treatment of 7 days of Phz tend to be restored to normal levels after 7 day treatment of DIEE. The recovery from hematoxic state to normal may probably be attributed to the anti-oxidant potential of the DIEE.

Though *Dillenia* and its species have been well documented to have anti-oxidant properties, studies were performed to ascertain whether the extract we are using possesses the activity¹⁹. In DPPH radical scavenging activity DIEE proved to be positive which corresponds to other studies performed. These studies delineates that the hematoprotective activity if DIEE is due to its radical scavenging activity.

Pertaining to its prime importance and activities, DI has been isolated and characterization of the active constituents has been done in various studies. Several compounds have been isolated from *Dillenia indica*, namely: lupeol, betulinaldehyde, stigmasterol, betulinic acid, beta-sitosterol, dillentin, betulin and other triterpenoids¹⁷-¹⁹. Presence of triterpenoids has been confirmed in Libermann-Buchard and Salkowski tests. It can be postulated that the hematoprotective activity of DIEE in our experiments may be due to Betulinic acid and related triterpenoids as this active compounds have been isolated from *Dillenia indica*, namely: lupeol, betulinaldehyde, stigmasterol, betulinic acid, beta-sitosterol, dillentin, betulin and other triterpenoids¹⁷-¹⁹. Presence of triterpenoids has been confirmed in Libermann-Buchard and Salkowski tests. It can be postulated that the hematoprotective activity of DIEE in our experiments may be due to Betulinic acid and related triterpenoids as this active compound is due to its radical scavenging activity.

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**Table 3**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hgb (g/dl)</th>
<th>RBC (X10¹²/L)</th>
<th>Hct (%)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dL)</th>
<th>TLC (X10¹²/L)</th>
<th>Platelet (X10¹²/L)</th>
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<tbody>
<tr>
<td>I</td>
<td>10.5±0.76</td>
<td>6.58±0.37</td>
<td>35.6±4.3</td>
<td>55.2±3.34</td>
<td>12.5±1.83</td>
<td>30.83±3.29</td>
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<td>400±69.29</td>
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<td>II(a)</td>
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<td>33±6.66</td>
<td>54.1±4.95</td>
<td>13±0.86</td>
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<td>12.86±1.42</td>
<td>380±72.97</td>
</tr>
<tr>
<td>II(b)</td>
<td>10.8±1.7³</td>
<td>6.2±0.54³</td>
<td>30.4±3.45</td>
<td>54.4±5.57</td>
<td>13.5±0.6</td>
<td>31.4±5.0³</td>
<td>16.4±5.67</td>
<td>498±117.39</td>
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<tr>
<td>III(a)</td>
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<td>6.9±1.21³</td>
<td>31.2±2.3</td>
<td>62±5.32</td>
<td>14.8±1.47</td>
<td>34.8±2.4³</td>
<td>10.6±2.0⁸</td>
<td>440±83.27</td>
</tr>
<tr>
<td>III(b)</td>
<td>13±1.7³</td>
<td>5.9±0.95³</td>
<td>36.9±4.38</td>
<td>51.3±7.83</td>
<td>13.9±0.96</td>
<td>37.8±5.3³</td>
<td>11.4±2.1³</td>
<td>465±101.6³</td>
</tr>
<tr>
<td>IV(a)</td>
<td>12.5±2.3³</td>
<td>6.8±0.4²</td>
<td>30.7±3.01</td>
<td>59.4±3.41</td>
<td>14.1±0.8⁴</td>
<td>26.4±5.07³</td>
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<td>418±60.18³</td>
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<td>IV(b)</td>
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<td>37.1±3.5</td>
<td>59.7±5.7³</td>
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<td>29.4±2.7³</td>
<td>12.4±1.8³</td>
<td>412±65.98³</td>
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</table>

Fig. 2- Graphical representation of fold change of mRNA expression levels as observed in q-PCR for SOD-2 (a) and SOD-3 (b). Data presented as Mean±SD; *** p, 0.001; **p, 0.005.

Fig. 3- Graphical representation of fold change of mRNA expression levels as observed in q-PCR for Nfer-1(a) and Nfer-2 (b). Data presented as Mean±SD; **p, 0.005; *p, 0.05.
constituent has been reported to exert similar effects as observed in present study. Anti-oxidant enzymes such as, SODs, Catalase, Glutathione, Heme-oxygenase, Glutathione peroxidase, Nfer, NADPH oxidase have an important role in maintaining the redox balances in biological system. This balance is quite crucial for hematopoietic system as blood and its components are very susceptible towards any changes in levels free radicals. To maintain this redox balances these enzymes and enzyme systems serves intrinsic combat mechanism. The prime function is being protection of system against oxidative stress. Thus, obtained balance between pro and anti-oxidant intrinsic mechanisms leads to the general and normal functioning of the system.

Superoxide dismutases (SODs) are metallo proteins that protect organisms from toxic reactive oxygen species by catalysing the conversion of superoxide anion to hydrogen peroxide and molecular oxygen. There are three major SODs in the mammalian system. SOD1, SOD2 and SOD3 – amongst which SOD2 and SOD3 are of prime importance to hematopoietic system. Very little work has been performed on SOD3 but it shows to be of integral importance in mammalian defence against oxidative injury especially in the context of Nfer-2 mediated protection.

Nfer has been known to protect cells from exogenous xenobiotics, toxicants and other deleterious effects by stimulating other anti-oxidant genes. Nuclear erythroid 2 p45-related factor 2 (Nfer-2) is a redox-sensitive, basic leucine zipper protein that regulates the transcription of several antioxidant genes and are essential for normal differentiation of erythroid precursors. Nfer-1 and 2 are omnipresent and also have role to play in the regulation of intrinsic anti-oxidant defence machinery.

The present study work reveals that the protective effects of Dillenia indica were imparted due to its potential to enhance the enzymes involved in the defence against the oxidative stress and it does not exert any toxic effects as studied in 54 day sub-chronic toxicity studies.

The study proves the positive effects of DIEE in mammalian system. DIEE is found to have hematoprotective effects in Phenylhydrazine based Sprague-dawley rat model. DIEE showed anti-oxidative potential in DPPH radical scavenging assay. DIEE and its fractions exhibited hematoprotective activity on isolated erythrocytes. Phytochemical analysis of the active n-hexane fraction revealed the presence of triterpenoids. Presence of triterpenoids in hexane fraction and hematoprotective activity can be co-related but requires further estimation. In mechanistic studies, it was unveiled that DIEE imparts its beneficial effects via activation of anti-oxidant enzymes. The results obtained make it encouraging studying DI further as a potential agent against hematotoxic stress which is manifested as ADRs and encountered in response to various therapeutic regimes. Additional studies especially clinical ones are to be performed in ADR manifested patients to certify the finding observed in vivo. More studies might be employed with larger groups with additional doses to make the outcome more prominent and comprehensive.

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