HEPATOPROTETECTIVE ACTIVITY OF HERBAL PREPARATION (HP-4) AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN MICE

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ABSTRACT
Excessively formed free radicals or oxidants cause an imbalance between oxidants and antioxidants in oxidative stress which is the cause of diseases like cancer, diabetes and liver diseases. Liver detoxifies paracetamol but overdoses causes hepatotoxicity. In the present study Herbal Preparation or HP-4 a combination of 80% alcoholic extract containing leaves of Aloe vera, Bacopa monniera, Moringa oleifera and rhizome of Zingiber officinale utilized to find its hepatoprotective activity in paracetamol induced hepatotoxicity. Group I or Control group of mice was fed on distilled water p.o. for 9 days. On the fifth day of the experiment Groups II, III, IV, V were induced with hepatotoxicant that is Paracetamol at dosage 750mg/kg p.o. For 5 days prior to and 4 days after toxicant treatment Group III and IV were fed HP-4 at 250 mg/kg and 500mg/kg p.o., whereas Group III was fed Silymarin 200mg/kg p.o. The hepatic marker enzymes such as alanine transaminase (ALT), aspartate Transaminase (AST), alkaline Phosphatase (ALP), gamma glutamyl transaminise (γGT), lactate dehydrogenase (LDH) and oxidative stress marker thiorbarbituric acid reactive substances (TBARS) showed increased levels in toxicant group. Hepatic antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and reduced glutathione (GSH) showed decrease levels in the toxicant group. Hepatoprotective activity of HP-4 was comparable to standard drug Silymarin hepatic marker enzymes levels were attenuated and hepatic antioxidant enzymes levels were increased. Histopathological studies and observations further supported the hepatoprotective nature of HP-4. The study provides evidence that the phytochemicals in HP-4 alone and in combination function in a synergistic manner in providing protection against Paracetamol induced hepatotoxicity in mice.

Key Words: Free Radicals, Antioxidants, Oxidative Stress, Hepatotoxicant, Paracetamol, Hepatic Marker Enzymes, Phytochemicals

INTRODUCTION
The hyper-physiological burden of free radicals causes imbalance in homeostatic phenomena between oxidants and antioxidants in the body. This imbalance leads to oxidative stress which is the root cause of aging; diseases like diabetes, cancer and liver damage (James et al., 2003). Liver is an organ of prime importance, which plays an essential role in the metabolism, in the detoxication of toxic compounds entering the body or formed in the body (Rajesh et al., 2009). The liver is expected not only to perform physiological functions but also protect against the hazards of harmful drugs and chemicals (Rajesh and Latha, 2004; Gujarati et al., 2007). Paracetamol is widely used over -the -counter drug for analgesic and anti-pyretic effects (Prescot, 2000). Paracetamol or Acetaminophen produces acute liver damage if overdoses are consumed (Lee, 2004). Paracetamol induced hepatic failure is the second leading cause of liver transplantation and accounts to considerable levels of morbidity and mortality (Olaleye et al., 2006). Under normal conditions, paracetamol is primarily metabolized in the liver by glucuronidation and sulfation (Nelson, 1995). Paracetamol induced hepatic failure is the second leading cause of liver transplantation and accounts to considerable levels of morbidity and mortality (Olaleye et al., 2006). The hepatotoxicity is the result of the formation of a toxic metabolite N-acetyl-p-benzoquinoneimine (NAPQI) by cytochrome P 450 2 E 1 that can induce a dose -dependent depletion of intracellular glutathione and...
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perturbations of calcium homeostasis (Gardner et al., 2003). Excess of NAPQI causes oxidative stress and binds covalently to liver proteins (Nelson, 1995). Although the precise mechanism by which paracetamol causes cell injury is still unknown; it is suggested that mitochondria may play an important role in the paracetamol induced liver deaths (Ferret et al., 2001). In recent years, the usage of herbal drugs for the treatment of liver diseases has increased all over the world (Girish and Pradhan, 2008). The herbal drugs are believed to be harmless and free from serious adverse reactions; as they are obtained from nature; and are easily available. Also due to limited therapeutic options and disappointing therapeutic success of modern medicine has increased usage of alternative medicine including herbal preparation (Stickel and Schuppan, 2007). Aloe vera (Liliaceae) is a cactus like plant with green, dagger shaped leaves that are fleshy and filled with clear viscous gel (Singh et al., 2010). The colourless, mucilaginous gel is obtained from the parenchymatous cells in the fresh leaves of Aloe vera. Studies have confirmed the efficacy of the water extract of Aloe vera against CCl₄ induced liver damage as indicated by increase in bile flow and bile solids suggestive of stimulation of secretory activity of liver cells. The hepatoprotective activity was attributed to preserving the metabolizing enzymes of the liver through an antioxidant activity (Alves et al., 2004). Bacopa monnieri Linn (Scrophulariaceae) commonly known as “Brahmi” is a component of several popular drugs of Ayurvedic system of medicine. The plant is reported to contain tetracyclic triterpenoids, saponins, bacosides A and B, hersaponin, alkaloids viz herpestine brahmine and flavonoids (Tripathi et al., 1996). Saponins of Bacopa monnieri were shown to have hepatoprotective activity in paracetamol model due to their antioxidant action (Ghosh et al., 2007). Moringa oleifera (Family Moringaceae) is a tree cultivated for different purposes such as medicine, vegetable, spice for cooking and cosmetic oil. It is commonly known as ‘drumstick’ (Goyal et al., 2007). The methanolic and chloroform extracts of leaves of Moringa oleifera have shown significant hepatoprotection against CCl₄ induced hepatotoxicity (Hamzaa, 2010). Zingiber officinale or ginger belongs to Zingiberaceae family. The rhizome of ginger has been used as medicine from Vedic period and is called “maha -aushadhi” means ‘the great medicine’ (Polassa and Nirmala, 2003). Ginger is also having significant hepatoprotective activity. The bromobenzene (BB) induced hepatotoxicity is counteracted by its reactive metabolites (El-Sharaky et al., 2009). In the present study, the hepatoprotective activity of herbal preparation (HP-4) containing 80% alcoholic extract of Aloe vera, Bacopa monnieri, Moringa oleifera leaves and rhizome of Zingiber officinale taken in equal proportion, was assessed against Silymarin, a standard drug in paracetamol induced hepatotoxicity in mice.

MATERIALS AND METHODS
The leaves of Aloe vera, Bacopa monnieri and Moringa oleifera and also rhizome of Zingiber officinale were collected from Loni, Maharashtra. The herbs were identified by a Professor of Botany, Loni. The leaves & rhizome were shade dried for 4-6 weeks and powdered finely in a mixture and sieved twice to obtain a fine powder. 100 gm dried powder of leaves of each Aloe vera, Bacopa monnieri, Moringa oleifera and rhizome of Zingiber officinale were separately extracted with Soxhlet extractor using 80% alcohol till solvent was colourless. The extract was dried till constant weight was obtained. 25mg of each extract was mixed together and dissolved in 10 ml methanol, boiled in water bath for 5 minutes, cooled and centrifuged at 4000 rpm for 10 minutes. The clear supernatant obtained was labeled as Herbal Preparation (HP-4) as reported earlier (Padmanabhan and Jangle, 2012a).

Animals: Swiss albino male mice weighing 25-30 g bred in Animal Resources Centre for Medical Research, PIMS Loni were used. The animals were allowed standard food pellets and water ad libitum. They were maintained in standard laboratory conditions (12:12 hr L: D cycle and 25 ± 2 °C). The study protocol was approved by “The Institute Animal Ethical Committee” PIMS /AH/215 /2011 PIMS Loni and CPCSEA Reg No 366/01/a/CPCSEA.

Study Design: 20 male mice of weight 25-30 g were divided into 5 Groups of 4 animals each.

Group I Normal Control: The animals received distilled water passed orally for 9 days.
Group II Toxicant Paracetamol Group: The animals received a single dose of paracetamol 750mg/kg p.o. on the first day.

Group III: Standard Silymarin Group: The animals received Silymarin 200mg/kg p.o. for first 5 days. Single dose of Paracetamol 750mg/kg p.o. on 5th day. Further Silymarin of same dosage was continued for next 4 days.

Group IV Toxicant + HP-4 250mg/kg Group: HP-4 250mg/kg was p.o for 5 days. Single dose of Paracetamol 750mg/kg p.o. on 5th day. Further HP-4 of 250mg/kg dosage was continued for next 4 days.

Group V Toxicant + HP-4 500mg/kg Group: HP-4 500mg/kg was p.o for 5 days. Single dose of Paracetamol 750mg/kg p.o on 5th day. Further HP-4 of 500mg/kg dosage continued for next 4 days.

Biochemical and Histopathological Parameters: On the 10th day after overnight fast the blood was collected from retro-orbital plexus. The blood was allowed to clot and centrifuged (Remi –R 8C Centrifuge) at 2500 rpm for 10 minutes. The serum was separated and used for assay of alanine transaminase (ALT) EC 2.6.1.2 (Bradley et al., 1972) aspartate transaminase (AST) EC 2.6.1.1 Wolf et al., (1972), alkaline phosphatase (ALP) EC 3.1.3.1 (Young et al., 1972) γ glutamyl transferase (γ GGT) EC 2.3.2.2 (Persijn et al., 1976) and lactate dehydrogenase (LDH)EC 1.1.1.27 (Lum and Gambino, 1974) by using standard methods using enzyme assay kits (Transasia Bio –medicals Ltd Kit for ALT,AST, LDH and Accurex Biomedicals Ltd Kit for γ GGT & ALP. The enzyme assays were performed on a semiautoanalyzer ERBA Chem 7. The remaining part of the liver was weighed and was homogenized in phosphate buffer 0.2M, pH =7.4 (Lal et al., 2007). The 10% homogenized liver tissue was made using a tissue homogenizer (MC Dalal & Co). The supernatant obtained after centrifuging at 4000rpm for 10 minutes was used for estimation of SOD, GPx, GR, TBARS & GSH. Total proteins in the supernatant were estimated by Biuret Method (Flack and Woollen, 1984).

**Determination of superoxide dismutase (SOD) EC 1.15.1.1:** SOD was determined by the method of (Marklund and Marklund, 1974). The SOD activities of the supernatant of tissue homogenate were estimated by measuring the % inhibition of the pyrogallol autoxidation by SOD. 2.5 ml of Tris Buffer pH 8.2, 0.05M, 0.5 ml of 1mM EDTA, 0.5ml Pyrogallol 0.2mM freshly prepared were added to 50µl of the supernatant of tissue homogenate. OD at 420nm after 5 minutes was recorded. One unit of SOD was defined as the enzyme activity that inhibited the autoxidation of pyrogallol by 50 percent.

**Determination of glutathione peroxidase (GPx) EC 1.11.1.9:** GPx activity was measured by the method described by (Rotruick et al., 1973). Briefly, reaction mixture contained 0.2ml of 0.4 M Tris HCL buffer pH 7.0, 0.1ml of 10mM sodium azide, 0.2 ml of the supernatant of liver tissue homogenate, 0.2 ml glutathione 60mg%, 0.1ml of 0.2M H₂O₂. The contents were incubated at 37 °C for 10 minutes. The reaction was arrested by 0.5 ml of 10% TCA and centrifuged. Supernatant was assayed for glutathione content by using Ellmans reagent (19.8mg of 5, 5’ dithiobis-(2 nitro benzoic acid) DTNB.

**Determination of glutathione reductase (GR) EC 1.6.4.2:** activity was measured spectrophotometrically according to the method of (Calberg and Mannervick, 1985). The reaction mixture contained 1mM oxidized glutathione GSSG as substrate, 2mM NADPH and tissue homogenate in phosphate buffer (pH=7.4). The decrease in absorbance at 340nm in terms of NADPH oxidation was measured. One unit of enzyme activity is defined as 1nmol of NADPH oxidized in one minute per mg protein.

**Determination of thiobarbituric acid reactive substances (TBARS):** TBARS in tissues was estimated by the method of (Fraga et al., 1981).

To 0.5 ml of supernatant of tissue homogenate, 0.5 ml saline and 1.0 ml 10% TCA were added, mixed well and centrifuged at 3000 rpm for 20 minutes. To 1.0 ml of protein free supernatant, 0.25 ml Thiobarbituric acid (TBA) reagent was added, the contents were mixed well and boiled for one hour at 95 °C. The tubes were then cooled to room temperature under running tap water and absorbance was measured at 532 nm.
Determination of Reduced Glutathione (GSH): Spectrophotometric quantification of reduced glutathione (GSH) has been carried out using 5, 5′-dithiobis-(2 nitro benzoic acid) DTNB reagent according to method proposed by (Moron et al., 1979). Briefly, 200 µl of supernatant of tissue homogenate (10%) was added to 800 µl distilled water and then 2 ml of sodium phosphate –EDTA buffer (0.1 M of sodium phosphate, 0.005 M EDTA buffer pH 8.0) containing 0.6M DTNB were added. The optical density of the yellow coloured complex developed by the reaction of GSH and DTNB was measured at 412nm using a UV –vis –spectrophotometer.

Histopathology: The animals were sacrificed by cervical dislocation & liver was excised, washed with saline and dried with tissue paper. A part of the lobe of liver was transferred to Formasaline solution for histopathological studies. The liver tissues were processed for paraffin embedding and sections 5 µm thick were taken in a microtome. After staining with hematoxylin and eosin slides were examined under the microscope for histopathological changes (Bancroft and Cook, 1984).

RESULTS AND DISCUSSION

Table 1: Effect of Herbal Preparation HP-4 on the Liver Function Tests as a marker of liver damage in paracetamol (PCM) induced hepatotoxicity in mice

<table>
<thead>
<tr>
<th>S. No</th>
<th>ALT Units in IU/L</th>
<th>AST Units in IU/L</th>
<th>ALP Units in IU/L</th>
<th>LDH Units in IU/L</th>
<th>γGT Units in IU/L</th>
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<tbody>
<tr>
<td>Group I Control</td>
<td>38.36±3.90</td>
<td>29.60±2.89</td>
<td>89.61±8.49</td>
<td>302.12±32.61</td>
<td>3.94±0.48</td>
</tr>
<tr>
<td>Group II Paracetamol PCM (Toxicant) 750mg/kg</td>
<td>137.61±14.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>178.38±18.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>196.92±18.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1495.15±148.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.64±0.95&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III PCM+Silymarin 200mg/kg</td>
<td>39.38±3.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.17±3.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>91.62±10.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>485.05±49.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.90±0.42&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV PCM+250 mg/kg HP-4</td>
<td>90.32±10.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>86.19±8.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>114.69±11.98&lt;sup&gt;c&lt;/sup&gt;</td>
<td>797.42±80.91&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.34±0.88&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V PCM+500 mg/kg HP-4</td>
<td>43.44±5.47&lt;sup&gt;d&lt;/sup&gt;</td>
<td>34.15±3.66&lt;sup&gt;d&lt;/sup&gt;</td>
<td>93.97±9.16&lt;sup&gt;d&lt;/sup&gt;</td>
<td>806.12±82.70&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.29±0.55&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
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</table>

<sup>a</sup>p<0.05 Toxicant as compared to control, significantly increased
<sup>b</sup>p<0.05 Group III as compared to GroupII, significantly decreased
<sup>c</sup>p<0.05 Group IV as compared to GroupII, significantly decreased
<sup>d</sup>p<0.05 Group V as compared to GroupII, significantly decreased
### Table 2: Effect of Herbal Preparation HP-4 on liver antioxidant enzymes activities levels on Paracetamol (PCM) induced hepatotoxicity in mice

<table>
<thead>
<tr>
<th>S. No</th>
<th>SOD Units/100mg protein</th>
<th>GPx Nanomoles utilized/mg/min</th>
<th>GR Nanomoles NADPH/100mg protein</th>
</tr>
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<tbody>
<tr>
<td>Group I Control</td>
<td>1.92 ± 0.20</td>
<td>41.06 ± 4.89</td>
<td>4.24 ± 0.44</td>
</tr>
<tr>
<td>Group II Paracetamol PCM (Toxicant) 750mg/kg</td>
<td>1.40 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.68 ± 3.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.81 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III PCM+ Silymarin 200mg/kg</td>
<td>1.84 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.14 ± 4.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.69 ± 0.81&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV PCM+250mg/kg HP-4</td>
<td>1.80 ± 0.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40.51 ± 4.48&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.46 ± 0.73&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V PCM+500mg/kg HP-4</td>
<td>1.82 ± 0.19&lt;sup&gt;d&lt;/sup&gt;</td>
<td>47.61 ± 4.07&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.73 ± 0.84&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> p<0.05 Toxicant as compared to control, significantly increased  
<sup>b</sup> p<0.05 Group III as compared to GroupII, significantly decreased  
<sup>c</sup> p<0.05 Group IV as compared to GroupII, significantly decreased  
<sup>d</sup> p<0.05 Group V as compared to GroupII, significantly decreased

### Table 3: Effect of Herbal Preparation HP-4 on liver weight, total proteins, TBARS and Reduced GSH on Paracetamol hepatotoxicity in mice

<table>
<thead>
<tr>
<th>S. No</th>
<th>Wt of the liver In grams</th>
<th>TBARS nanomoles/100mg protein</th>
<th>Reduced GSH mg GSH/100mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I Control</td>
<td>1.690 ± 0.17</td>
<td>8.49 ± 0.82</td>
<td>2.29 ± 0.27</td>
</tr>
<tr>
<td>Group II Paracetamol PCM (Toxicant) 750mg/kg</td>
<td>1.821 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.36 ± 7.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.96 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III PCM+ Silymarin 200mg/kg</td>
<td>1.659 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.18 ± 3.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.12 ± 0.20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV PCM+250mg/kg HP-4</td>
<td>1.527 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>39.13 ± 3.80&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.69 ± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V PCM+500mg/kg HP-4</td>
<td>1.463 ± 0.15&lt;sup&gt;d&lt;/sup&gt;</td>
<td>48.00 ± 4.79&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.03 ± 0.24&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> p<0.05 Toxicant as compared to control, significantly increased  
<sup>b</sup> p<0.05 Group III as compared to GroupII, significantly decreased  
<sup>c</sup> p<0.05 Group IV as compared to GroupII, significantly decreased  
<sup>d</sup> p<0.05 Group V as compared to GroupII, significantly decreased
Activities of serum AST, ALT, ALP, γ GGT and LDH (marker enzymes for liver damage) were markedly elevated in paracetamol treated animals compared to normal control mice, indicating liver damage.
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Administration of herbal preparation HP-4 at doses of 250 and 500 mg/kg remarkably prevented paracetamol induced elevation of serum AST, ALT, ALP, γ GGT, and LDH in Table 1. Since oxidative stress contributes to the development of paracetamol induced hepatotoxicity the levels of liver antioxidant enzymes SOD, GPx, GR were measured. Values for the antioxidant enzymes were reduced in the Toxicant Group as compared to Normal Control. Treatment with both dosages of HP-4 significantly raised the antioxidant enzyme levels as compared to the mice treated with paracetamol. Results are depicted in Table 2. TBARS level increases whereas Reduced GSH levels decreases in Toxicant Group as compared to Normal Control. These results are shown in Table 3. In the present study, the levels of serum enzymes ALT, AST, γ GGT, LDH were increased reflecting the hepatocellular damage due to paracetamol. The herbal preparation HP-4 could however lower the ALT, AST, γ GGT, LDH in these paracetamol intoxicated animals. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotman and Lawhorn, 1978). Serum ALP are related to the function of hepatic cell, increased serum levels is due to increased synthesis, in the presence of increasing biliary pressure. Treatment with HP-4 can be considered as an expression of functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchymal cells. Effective control of alkaline phosphatase points towards an early improvement in the secretory mechanisms of the hepatic cells (Muriel et al., 1992). The protective effect maybe the result of stabilization of plasma membrane, thereby preserving the structural integrity of cell as well as the repair of hepatic damage caused by paracetamol (Pari and Murugan, 2004). SOD has been reported as the most important enzyme in the enzymatic antioxidant defense system. It scavenges superoxide anion to form hydrogen peroxide; diminishing toxic effects of superoxide anion radical (Murugesh et al., 2005). In the present study SOD levels reported to have decreased in livers of the paracetamol treated mice and thereafter HP-4 and standard drug Silymarin causes significant increase in the SOD levels; thereby decreasing free radical damage and improve the activities of antioxidant enzymes. Such observation was reported by (Bhanwara et al., 2004) using A. indica extract in paracetamol induced toxicity in rats. In our study decrease a GPx and GR activity in animals administered paracetamol is noted. Such decrease in activity of GPx & GR was reported by (Kumar et al., 2005) (40) in paracetamol induced hepatotoxicity in rats. It was reported by them that β-carotene can act as an antihepatotoxic agent. HP-4 treatment has restored to some extent the GPx levels and GR. The antioxidant nature of β-carotene and HP-4 may be responsible for this. The results were compared with standard drug Silymarin. Silymarin is known for its hepatoprotective action and is widely used in various liver ailments (Murugesh et al., 2005). Lipid peroxidation has been postulated as being the destructive process in the liver injury due to paracetamol administration (Singh et al., 2011). The present study depicts elevation of thiobarbituric acid reactive substances (TBARS) in liver of mice treated with paracetamol. The increase in TBARS levels in liver suggests lipid peroxidation leading to tissue damage and failure of the antioxidant defense mechanisms; which prevents the formation of excessive free radicals. Treatment with HP-4 significantly reversed these changes. Hence it is possible that the mechanism of hepatoprotection of HP-4 is due to antioxidant effect. In vitro antioxidant effect of HP-4 in various experimental models was reported by authors (Padmanabhan and Jangle, 2012a). HP-4 is previously reported to have phenolic compounds, flavonoids, and flavonols. Moreover the HP-4 was reported to have synergistic antioxidant effect on DPPH scavenging activity (Padmanabhan and Jangle, 2012b). The protection to hepatic injury by paracetamol maybe due to the presence of various phytochemicals having antioxidant action. The TBARS result of Silymarin group was significant and comparable to that of HP-4 activity. GSH is one of the most abundant tripeptide, non-enzymatic biological antioxidants present in the liver. It removes free radical species such as, hydrogen peroxide, superoxide anion, alkoxy radicals and maintenance of membrane protein thiols as substrate for glutathione peroxidase and glutathione – S – transferase and toxic effects of toxicants are manifested by its depletion (Prakash et al., 2001). In our present study decline in GSH content in the liver of paracetamol intoxicated mice and its subsequent return to normalcy on administration of HP-4 as well as standard drug Silymarin also reveal the anti-lipid peroxidative effect of HP-4. Similar to observation was reported by.
Kuriakose et al., using Aphanizomenon flos-aquae in paracetamol intoxication in rats (Kuriakose et al., 2010). Paracetamol is converted to its reactive metabolite N-Acetyl-p-benzoquioneimine (NAPQI) by specific isoenzyme of cytochrome P-450. NAPQI can rapidly react with glutathione (GSH) and can lead to 90% total hepatic GSH depletion in cells and mitochondria. This leads to hepatocellular death and mitochondrial dysfunction. In addition NAPQI increases reactive oxygen species (ROS) and reactive nitrogen species (RNS). Excess levels of ROS and RNS attack the biological membranes such as DNA, proteins and phospholipids which leads to lipid peroxidation, nitration of tyrosine and depletion of antioxidant enzymes SOD and GPx that further results in oxidative stress. NAPQI an also induce DNA strand breaks and promote apoptosis, necrosis (Fraga and Oteiza, 2002). HP-4 is a mixture of Aloe vera, Bacopa monnieri, Moringa oleifera and Zingiber officinale. Aloe vera is reported to have hepatoprotective affect against CCl₄ induced hepatotoxicity which maybe due to antioxidant action of phytochemicals (Chandan et al., 2006). Cytoprotective effects in vitro by A.vera in paracetamol mediated oxidative stress in rat liver slices reported by (Patil, 2010). According to Ghosh et al., (2007), hepatoprotective effect of ethanolic extract of B. monnieri aerial parts in paracetamol toxicity was due to antioxidant activity of phenolics in the extract. They attributed the protective action on lipid peroxidation and at the same time the enhancing effects on cellular antioxidant defense contributing to the protection against oxidative damage in paracetamol induced hepatotoxicity. Hydroethanolic extract of different parts of Moringa oleifera could alleviate paracetamol induced hepatotoxicity in rats. The depleted levels of hepatic GSH, SOD, CAT & serum ALT could be restored and elevated level of hepatic MDA was decreased by administration of the extract. The authors attribute such beneficial effects due to antioxidant phytochemicals in the extract (Fakurazi et al., 2012). Noria et al., (2004) reported the protective effect of ethanol extract of Z.officinale on paracetamol induced hepatotoxicity in rats .The authors are of the opinion that the gingerol, the active ingredient of Z.officinale increases the level of glutathione which binds to the toxic metabolites of paracetamol such as NAPQI and increases its rate of excretion from body. Jagetia et al., (2003) reported that Z. officinale might inhibit the activity of cytochrome P 450 enzyme system which decreases the formation of NAPQI from ingested paracetamol. Ahmed et al., (2000) reported that Z. officinale was able to increase levels of glutathione and decrease lipid peroxidation due to antioxidant properties of Z.officinale. The histopathological studies also supported the protective properties of herbal preparation HP-4. Histological sections of liver showed centrilobular necrosis with inflammatory cell infiltration, ballooning degeneration of hepatocytes observed in toxicant group. The groups of animals treated with herbal preparation HP-4 and even standard drug Silymarin showed marked protective effects with decreased necrotic zones, decreased hepatocellular degeneration, decreased congestion and inflammatory cell infiltration. Since HP-4 is a consortium of Aloe vera, Bacopa monnieri, Moringa oleifera & Zingiber officinale the phytochemicals alone and their combination in synergistic way are exhibiting their hepatoprotective action as evidenced by biochemical and histopathological studies.

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