Chapter V

Hepatoprotective efficacy of *Carum carvi*, *Parmelia perlata* and Betaine against acetaminophen induced hepatic injury

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Betaine  
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1. Introduction

Drug induced liver toxicity is the most severe problem in the present time (Tujios and Fontana 2011). Liver being the central organ for the metabolic disposition of virtually all drugs and foreign substances which becomes the primary target for drug induced toxicity (Grattagliano 2009; Farrell 1994). Although, at therapeutic dose, drugs are usually metabolised without liver injury, but over administration of drugs can lead to the generation of reactive metabolites that can lead to liver toxicity.

Acetaminophen or paracetamol was discovered in Germany at the end of the 19\textsuperscript{th} century, but was not widely used until midway through the 20\textsuperscript{th} century. Acetaminophen is probably the most versatile and widely used analgesic and antipyretic drug worldwide (Rocha et al., 2005). Its potential hepatotoxicity was not suspected until the first clinical reports of severe and fatal liver damage following over dosage was reported by Davidson and Eastham in 1996. Acetaminophen taken in overdose resulted in hepatic and renal toxicities in men and in experimental animals (Vermeulen et al., 1992). Acetaminophen, N-acetyl-p-aminophenol (APAP), an efficient analgesic and anti-pyretic has an excellent safety profile in therapeutic doses, but hepatotoxicity can develop when consumed in overdoses. The major target organ in acetaminophen toxicity is the liver and the primary lesion is acute centrilobular hepatic necrosis (James et al., 1975). In adults the single acute threshold dose for severe liver damage is 150 to 250mg/kg but there is a marked individual variation in susceptibility.

When acetaminophen is ingested, it is mainly metabolized by the liver and excreted in the urine (Mitchell et al., 1973; Nelson, 1990). At therapeutic doses in humans, the major portion of acetaminophen is metabolized via Phase II conjugation and is converted to sulfate and glucuronide conjugates (approximately 30\% and 55\% respectively) by cytosolic sulfotransferases and microsomal glucuronosyltransferases. These conjugates are not believed to contribute to hepatotoxicity as they are easily excreted. In the presence of the cytochrome P450 monooxygenase system, a small portion (approximately 8-10\%) is metabolized to the electrophilic, toxic, reactive intermediate N-acetyl-p-benzoquinoneimine (NAPQI). NAPQI is then inactivated enzymatically (glutathione-S-transferases) or non-enzymatically (glutathione). The subsequent non-toxic cysteine and mercapturic acid conjugates (4\% each of the total acetaminophen dose) can be excreted in the urine (Jackson et al., 1984). However, at toxic doses of acetaminophen, an imbalance between bioactivation and detoxification occurs due to the large amount of NAPQI being generated as more acetaminophen is available to be metabolized via P450's to the reactive
metabolite. Under these conditions, the major detoxification pathway of NAPQI becomes saturated as GSH stores are depleted by as much as 75%, compromising the cellular defence mechanisms (Potter et al., 1974; Sipes and Gandolfi, 1991). The NAPQI can then attack nucleophilic sites on essential cellular macromolecules (Covalent binding), leading to cell death.

It is widely accepted that cytochrome P450’s are responsible for the formation of the reactive intermediate in the liver, as was first proposed by Mitchell and colleagues in 1973 (Mitchell et al., 1973). In experiments using mice and rats, it was discovered that pretreatment with cytochrome P450 inducers such as phenobarbital (PB) and 3-
rnethylcholanthrene (3-MC) increased the incidence and severity of hepatic necrosis (Mitchell et al., 1973; Potter et al., 1974). Cytochrome P450 inhibitors, cobaltous chloride and piperonyl butoxide, were found to decrease the amount of covalent binding and acetaminophen metabolism thus lessening the severity of the toxicity (Mitchell et al., 1973; Jollow et al., 1973). In addition, Potter and colleagues (1973) discovered that metabolite formation and covalent binding required the presence of oxygen and NADPH (P450 requirements) and that they were inhibited by the presence of carbon monoxide or antibodies raised against NADPH cytochrome c reductase.

It is clear that the metabolic pathway for acetaminophen involves phase 1 and 2 reactions, glutathione detoxification, and the formation of reactive intermediates, which disrupt cell macromolecules (Lee, 1995; Jos et al., 2001). The mechanism of acetaminophen induced liver injury has not been fully defined, however, it has been postulated that generation of free radicals (ROS) (Adamson and Harman 1993), transcription factor NF kappa B (Blazka et al., 1995a; Blazka et al., 1995b), nitric oxides (Gordon et al., 1986) and liver peroxides (Kamiyama, 1993) were the most important factors involved in acetaminophen-induced hepatic toxicity.

It is generally accepted that the ultimate form of hepatic damage caused by paracetamol is necrosis (Adams et al., 2001; Pierce et al., 2002; Knight and Jaeschke, 2002; Gujral et al., 2002). However, several reports have presented evidence for the occurrence of apoptosis in paracetamol induced hepatic damage. For instance, Ferret and co-workers have shown that caspase-3 and 9 activities were slightly increased in mice that were administered with a hepatotoxic dose of acetaminophen (Ferret et al., 2001), possibility of direct induction of apoptosis by the cytotoxic metabolite of paracetamol. N-acetyl-p-benzoquinone imine, additional factors or cytokines such as tumor necrosis factor have been implicated in paracetamol induced liver damage (Laskin et al., 1995; Gardner et al., 2002; Fiorucci et al., 2002).

In addition to the free radical damage, paracetamol has been found to activate signal transduction pathways involving transcription factors such as NF-κB and activator protein-1 (AP-1) (Chiu et al., 2003). These transcription factors are known to regulate the expression of genes controlling inflammatory mediators including nitric oxide synthase II, TNF-α, IL-1B, COX-2, each of which are associated with paracetamol induced hepatotoxicity (Laskin and Gardner 2003).

Studies are going on throughout the world for the search of protective molecules that would provide maximum protection against drug induced toxicities and practically very
little or no side effects would be exerted during their function in the body (Montilla et al., 2005; Mansour et al., 2006). A number of herbs are traditionally used in different countries during drug or toxin induced hepatic and renal disorders (El-Beshbishy, 2005). The present study was designed to evaluate the hepatoprotective efficacy of *Carum carvi*, *Parmelia perlata* and betaine against acetaminophen induced liver damage and to elucidate the alterations of various protein markers associated in the mechanisms of acetaminophen toxicity.

*Carum carvi* Linn, commonly known as caraway belongs to the family Umbelliferae, is a globally distributed spice with a history as a medicinal plant since ancient times (Hartmans et al., 1995). The dried ripe fruits of the plant are used in folk medicine especially in the treatment of digestive disorders in both adults and infants (Reynolds et al., 1993, Thompson et al., 2002). The main constituents of *Carum carvi* are the volatile oils including carvone (40–60%), limonene, carveol, dihydrocarveol and thymol in addition to glycosides and flavonoids (Zheng et al., 1992; Matsumura et al., 2002). Experimental studies have shown that *Carum carvi* possesses, antispasmodic (Eddouks et al., 2004), antiulcerogenic (Khayyal et al., 2001), antibacterial (Singh et al., 2002), antitumor (Kamaleeswari et al., 2006), antiproliferative (Nakano et al., 1998), antioxidant (Kamaleeswari et al., 2006), antihyperglycemic (Eddouks et al., 2004), antihyperlipidaemic (Lemhadri et al., 2006) and diuretic (Lahlou et al., 2007) activities.

*Parmelia perlata* (lichen) commonly known as Charila or Stone flower in India, belongs to family Parmeliaceae. Parmelia perlata exhibited strong antioxidant, antibiotic and antidiabetic activities (Patil et al., 2011). It is usually used as a spice to enhance the taste and flavor of the foods. Because of its medicinal properties it is also useful to treat sores, boils, inflammations, seminal weakness, and amenorrhoea (Warrier et al., 2002). It is important to note that at present, various herbal preparations used to treat seminal weakness and skin creams for wound healing contain *Parmelia perlata* as major component.

Betaine (N,N,N-trimethyl glycine), is widely distributed in animals, plants, microorganisms and rich dietary sources including seafood especially marine invertebrates, wheat germ or bran, sugar beets and spinach (Craig, 2004). It has been demonstrated that betaine has two important roles in the biological system. First, Betaine acts as an osmolyte, protects cells, proteins and enzymes from environmental stress for e.g., low water, high salinity, or extreme temperature and second it serves as a methyl group donor in various vital cellular pathways (Park and Garrow, 1999). Betaine prevents and cure ethanol hepatic steatosis (Barak et al., 1997), cirrhosis in rats (Webster, 1942; Best et al., 1969), mobilize hepatic cholesterol and phospholipids in rats fed a high cholesterol diet (Sugiyama et al.,
1986), treat hyperlipidemia (Turpin, 1985; Odle, 1996). Moreover, it has been observed that betaine suppresses the adverse effects of Homocystinuria, hereditary homozygous genetic dysfunction (Ogier de Baulny, 1998). Recently, Ozturk et al., unravels nephroprotective efficacy of betaine (Ozturk et al., 2003). Now a day, betaine is used as an essential dietary supplement.

On the basis of the above considerations, the present study was conducted to evaluate the antioxidant potential and chemopreventive efficacy of *Carum carvi*, *Parmelia perlata* and betaine against acetaminophen induced hepatotoxicity and to elucidate their role in the expression of NF kappa B, TNF-alfa and caspases in liver tissue.

2. Treatment regimen for acetaminophen induced liver toxicity and its modulation by *Carum carvi*, *Parmelia perlata* and Betaine

In this study, thirty female Wistar rats were randomly allocated to 5 groups of 6 rats each. Group I as control, received only distilled water orally. Groups III and IV received modulator (*Carum carvi*, *Parmelia perlata*, Betaine) at doses D1 & D2 respectively by gavage once daily for 7 days. Groups II, III and IV have given acetaminophen at a dose of 1g/kg body weight from 5th to 7th days orally. Group V has given higher dose (D2) of only modulator for 7 days orally.

After 7 days, the animals were sacrificed by cervical dislocation. Blood and liver samples were taken for various biochemical and molecular parameters.
3. Results

3.1. Study 1: Amelioration of acetaminophen induced liver toxicity by *Carum carvi*

3.1.1. Effect of *Carum carvi* on hepatic microsomal Cytochrome P450 (Table 1)

In the present study we found that in the acetaminophen treated group (28.64 ± 0.63) there is significant elevation in microsomal cytochrome P450 content as compared with the control group (13.91 ± 0.83) activity. Treatment of rats with *Carum carvi* at both doses (D1: 18.41 ± 0.45, p<0.01; D2: 14.21 ± 0.87, p<0.001) significantly restored the level of paracetamol metabolising enzyme (cytochrome P450) as compared with acetaminophen treated group. Animals treated with only *Carum carvi* showed no change in the Cytochrome p450 level as compared with the control group.

3.1.2. Treatment with *C. carvi* prevents depletion in the GSH level

Reduced glutathione (GSH) is the most important intra-cellular reductant that is involved in the catalysis, metabolism and transport of acetaminophen. Acetaminophen induced hepatotoxicity is associated with the depletion of GSH. Effect of *Carum carvi* on hepatic GSH levels was monitored. The level of GSH was decreased significantly (0.031±0.004, p<0.001) in paracetamol treated group as compared to respective control group (0.056 ± 0.01). Pretreatment with *C. carvi* showed significant (D1: p<0.01 and D2: p<0.001) elevation of the depleted GSH level (D1: 0.046 ± 0.004; D2: 0.052 ± 0.005) as compared to paracetamol treated group (Table 1). No significant changes in GSH level were observed in the group treated with only *C. carvi* as compared to the particular control groups.

3.1.3. Quinone reductase activity (QR) and Glutathione-S- transferase (GST) activity

Induction of phase II enzymes like quinone reductase and GST may increase resistance against acetaminophen induced hepatotoxicity. Treatment of *Carum carvi* extract at both doses (D1 & D2) resulted in the significant (P<0.001) elevation (D1 = 464.15 ± 39.82, D2 = 510.7 ± 49.07) in QR activity as compared with the acetaminophen treated group (350.17 ± 46.5). A significant decline in the GST activity (33.25 ± 5.8, p<0.001) was also observed in paracetamol treated group compared to the control (57.4 ± 7.5) group. A week’s treatment with *C. carvi* at both doses D1 and D2 elevated the GST activity significantly (D1: 45.3 ± 4.8, P<0.01; D2: 55.4 ± 7.01, p<0.001) as compared with the paracetamol treated group (Table 1).
3.1.4. Catalase (CAT) activity
Catalase activities in liver tissue of all experimental animals revealed, there was significant (p<0.001) depletion in catalase activity (29.41 ± 2.74) in paracetamol treated group compared to the untreated control (42.27 ± 1.29). Treatment with C. carvi at both doses (D1 & D2) elevated the CAT activity significantly (D1: 38.4 ± 2.6, p<0.05; D2: 41.24 ± 2.1, p<0.01). C. carvi alone did not show any significant difference as compared to control group (Table 1).

3.1.5. Glutathione peroxidase (GPx) activity
Rats treated with paracetamol showed a significant reduction (p<0.001) in the activity of GPx (138.4 ± 16.8) when compared with control animals (242.8 ± 19.5). However, significant restoration of GPx activity was observed in rats treated with C. carvi (D1= 198.6, p<0.01; D2= 232.1 ± 32.4, P<0.01). No significant difference was found in the only C. carvi treated group compared to the control (Table 1).

3.1.6. Effect of Carum carvi on hepatic membrane damage (LPO)
MDA formation is the manifestation of Lipid peroxidation hence measurement of its level is important marker to study cellular toxicity. Acetaminophen treatment caused significant increase (14.03 ± 1.15, p<0.001) in the level of MDA formation as compared with the control group (6.7 ± 0.19). Carum carvi were significantly effective in lowering down this acetaminophen induced lipid peroxidation at dose D1 (9.8 ± 0.7, p<0.01) and D2 (7.5 ± 0.5, P<0.001). Only Carum carvi group showed no significant change as compared with control animals (Table 1).

3.1.7. Elevated Level of serum liver toxicity markers as a result of acetaminophen exposure, restored by Carum carvi.
Over dose toxicity of acetaminophen is associated with fatal hepatic necrosis in experimental animals and humans. Leakage of cytosolic enzymes into the serum is a useful early quantitative marker for acetaminophen-induced hepatic damage indicating cell membrane disruption. In the present study, acetaminophen caused steep rise in the level of these toxicity parameters viz, AST (61%, p<0.001), ALT (86%, p<0.001) and LDH (63%, p<0.001) as compared with control group. Treatment of Carum carvi showed significant protection in bringing back these elevated levels of serum toxicity markers by 30% (p<0.01), 45% (p<0.05) and 36% (p<0.001) at dose D1 and 55% (p<0.001), 75% (p<0.01) and 57% (p<0.001) at dose D2 respectively as compared with the paracetamol treated
group confirming membrane stabilization and antioxidant properties of *Carum carvi* (Table 2).

### 3.1.8. Effect of *Carum carvi* treatment on hepatic TNF-α level

TNF-α levels in the hepatic tissue of the acetaminophen treated rats was significantly elevated (p<0.001) by 32% as compared with control group. *Carum carvi* administration showed a significant reduction in the hepatic TNF-α levels at both doses D1 (p<0.01) and D2 (p<0.01) by 24% and 34% respectively as compared with the acetaminophen treated group, whereas no significant change in the TNF-α levels were found in only D2 group as compared to control group (Figure 2).

### 3.1.9. Effect of *Carum carvi* administration on caspase 3, 7 and 9 activities

Figure 1 shows the expression of caspase 3, 7 and 9 in liver tissue of rats. Acetaminophen treated group shows a significant elevation of caspase 3, 7 and 9 by 356%, 355% and 185% respectively as compared to control group. This elevation in caspase activities is significantly depleted by *Carum carvi* administration. Caspase 3 activity was depleted by 254.6% (p<0.001) and 345% (p<0.001), caspase 7 activity by 260% (p<0.05) and 298% (p<0.01) and caspase 9 by 185% (p<0.01) and 218% (p<0.001) at doses D1 and D2 respectively, as compared with only acetaminophen treated group. Animals treated with only high dose of *Carum carvi* did not show any significant change as compared to control group.

### 3.1.10. Histopathology of liver tissue (Figure 3)

Liver sections of rats of control group showing structural intactness and normal architecture. Whereas acetaminophen treated group (400x) is characterized by widespread areas of congestion and hemorrhage in the centrilobular and midzonal areas of the liver. Confluent necrosis involving hepatocytes in the centrilobular zone; bridging of areas of necrosis between centrilobular zones is common. *Carum carvi* treatment at dose D1 is characterized by moderate congestion and hemorrhage of the area around the centrilobular vein and extending into the midzonal cells; most lobules are affected. Areas of confluent necrosis limited to the liver cells surrounding the centrilobular vein. Minimal congestion and necrosis of single hepatocytes, limited to the area immediately around the centrilobular vein; many of the lobules not affected in the tissues of animals treated with high dose of *Carum carvi* (D2). Only Carum carvi treated group showed normal liver architecture (Figure 3).
Table 1: Oxidative stress and cytotoxicity markers of liver toxicity and modulation by *Carum carvi*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Acetaminophen</th>
<th><em>Carum carvi</em> D1 + Acetaminophen</th>
<th><em>Carum carvi</em> D2 + Acetaminophen</th>
<th>Only <em>Carum carvi</em> D2</th>
</tr>
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<tbody>
<tr>
<td><strong>CYP 450</strong> (nmol/mg protein)</td>
<td>13.91 ± 0.83</td>
<td>28.64 ± 0.63*</td>
<td>18.41 ± 0.45**</td>
<td>14.21 ± 0.87***</td>
<td>14.01 ± 0.68</td>
</tr>
<tr>
<td><strong>LPO</strong> (nmolMDA formed/g tissue)</td>
<td>6.7 ± 0.19</td>
<td>14.03 ± 1.15*</td>
<td>9.8 ± 0.7**</td>
<td>7.5 ± 0.5***</td>
<td>6.4 ± 0.5</td>
</tr>
<tr>
<td><strong>Catalase</strong> (nmol H₂O₂ consumed/min/mg protein)</td>
<td>42.27 ± 1.29</td>
<td>29.41 ± 2.74*</td>
<td>38.4 ± 2.6*</td>
<td>41.24 ± 2.1**</td>
<td>41.39 ± 3.3</td>
</tr>
<tr>
<td><strong>Glutathione Peroxidase</strong> (nmol NADPH oxidized/min/mg protein)</td>
<td>242.8 ± 19.5</td>
<td>138.0 ± 16.8*</td>
<td>198.6 ± 25.4**</td>
<td>232.1 ± 32.4**</td>
<td>246.5 ± 21.8</td>
</tr>
<tr>
<td><strong>Glutathione reductase</strong> (nmol NADPH oxidized/min/mg protein)</td>
<td>318.8 ± 7.7</td>
<td>218.5 ± 7.6*</td>
<td>282.1 ± 9.2**</td>
<td>320.4 ± 11.5***</td>
<td>319.5 ± 8.7</td>
</tr>
<tr>
<td><strong>Reduced Glutathione</strong> (µmol GSH conjugate/g tissue) (+: SD)</td>
<td>0.056 ± 0.01*</td>
<td>0.031 ± 0.004*</td>
<td>0.046 ± 0.004**</td>
<td>0.052 ± 0.005***</td>
<td>0.058 ± 0.003</td>
</tr>
<tr>
<td><strong>Quinone reductase</strong> (nmol DCPIP reduced/min/mg protein)</td>
<td>602.12 ± 25.85</td>
<td>350.17 ± 46.5*</td>
<td>464.15 ± 39.82*</td>
<td>510.7 ± 49.07**</td>
<td>605.83 ± 50.95</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.E., n = 6
a P < 0.001 compared with the corresponding value for control group.
*P <0.05, **P <0.01 and ***P <0.001 compared with the corresponding value for acetaminophen treated group.
### Table 2: Serum Parameters

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Acetaminophen</th>
<th>Carum carvi D1 + Acetaminophen</th>
<th>Carum carvi D2 + Acetaminophen</th>
<th>Only Carum carvi D2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aspartate aminotransferase</strong> (AST or SGOT) (IU/L)</td>
<td>47.39 ± 3.44</td>
<td>76.5 ± 5.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.1 ± 5.4&lt;sup&gt;**&lt;/sup&gt;</td>
<td>50.47 ± 6.8&lt;sup&gt;***&lt;/sup&gt;</td>
<td>47.07 ± 3.8</td>
</tr>
<tr>
<td><strong>Alanine aminotransferase</strong> (ALT or SGPT) (IU/L)</td>
<td>10.36 ± 0.9</td>
<td>19.3 ± 2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.6 ± 1.33&lt;sup&gt;*&lt;/sup&gt;</td>
<td>11.5 ± 1.14&lt;sup&gt;**&lt;/sup&gt;</td>
<td>10.2 ± 1.6</td>
</tr>
<tr>
<td><strong>Lactate dehydrogenase</strong> (LDH) (nmol NADH oxidised/min/mg protein)</td>
<td>165.04 ± 16.3</td>
<td>268.3 ± 23.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>208.5 ± 24.6&lt;sup&gt;***&lt;/sup&gt;</td>
<td>174.2 ± 19.8&lt;sup&gt;***&lt;/sup&gt;</td>
<td>169.8 ± 21.1</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.E., n = 6

<sup>a</sup>P < 0.001 compared with the corresponding value for control group.

<sup>*</sup>P < 0.05, <sup>**</sup>P < 0.01 and <sup>***</sup>P < 0.001 compared with the corresponding value for acetaminophen treated group.

#### Figure 1: Effect of Carum carvi on Caspase activities.

Graph showing the effect of Carum carvi treatment on acetaminophen (paracetamol) induced caspase 3, 7 and 9.

Each value represents mean ± S.E., n = 6

### CC D1: Carum carvi 100mg/kg b. wt; CC D2: Carum carvi 200mg/kg b. wt.
Figure 2: Effect of *Carum carvi* on TNF-α expression in liver tissue

Representative graph showing the restoration of paracetamol induced TNF-α levels by *Carum carvi* in liver tissue.
Each value represents mean ± S.E., n = 6
CC D1: *Carum carvi* 100mg/kg b. wt; CC D2: *Carum carvi* 200mg/kg b. wt.
# P < 0.001 compared with the corresponding value for control group.
*P <0.05, **P <0.01 and ***P <0.001 compared with the corresponding value for acetaminophen treated group.
Figure 3: Effect of *Carum carvi* on histopathological architecture of liver tissue

Representative micrographs showing acetaminophen induced histopathological changes in liver tissue and effect of *Carum carvi* in the normalization of the anatomical architecture.

A) Liver section of rat control group showing structural intactness and normal architecture.
B) Acetaminophen treated group (400x).
C) Co-administration of *Carum carvi* (at a dose of 100mg/kg b.wt) with acetaminophen group. (400x).
D) Co-administration of *Carum carvi* (Dose 200mg/kg b Wt) (400x).
E) Slide showing liver histology of rats treated with only *Carum carvi* at a dose of 200mg/kg b wt. (400x).
3.2. Study 2: Amelioration of acetaminophen induced liver toxicity by *Parmelia perlata*

3.2.1. Effect of *Parmelia perlata* on hepatic microsomal Cytochrome P450

In the present study we found that in the acetaminophen treated group there is significant elevation (p<0.001) in microsomal cytochrome P450 content by 106% (28.64 ± 0.63) as compared with the control group (13.91 ± 0.83) activity. Treatment of rats with *Parmelia perlata* at both doses significantly restored the level of paracetamol metabolising enzyme (cytochrome P450) by 70% (D1: 18.81 ± 0.81, p<0.01) and 102% (D2: 14.43 ± 0.53, p<0.001) respectively as compared with acetaminophen treated group. Animals treated with only *Parmelia perlata* showed no change in the Cytochrome p450 level as compared with the control group (Table 3).

3.2.2. Treatment with *Parmelia perlata* prevents depletion in the GSH level

Acetaminophen induced hepatotoxicity is associated with the depletion of GSH. The level of GSH was decreased significantly in paracetamol treated group by 50% (0.024 ± 0.003, p<0.001) as compared to respective control group (0.048 ± 0.01). Pretreatment with *Parmelia perlata* showed significant elevation of the depleted GSH level by 40% at dose D1 (0.043 ± 0.007; p<0.01) and 48% at dose D2 (0.047 ± 0.006; p<0.001) as compared to paracetamol treated group. (Table 3) No significant changes in GSH level were observed in the group treated with only *Parmelia perlata* as compared to the particular control groups.

3.2.3. Glutathione-S-transferase (GST) activity

A significant decline in the GST activity by 36% was also observed in paracetamol treated group (37.5 ± 2.31, p<0.001) compared to the control (59.03 ± 3.62) group. Treatment with *Parmelia perlata* at both doses D1 and D2 elevates the GST activity significantly by 21% and 29% respectively (D1: 50.05 ± 2.65, P<0.05; D2: 54.8 ± 1.92, p<0.01) as compared with the paracetamol treated group. Whereas no significant change in the GST activity was observed in animals treated with only *Parmelia perlata* as compared with control group (Table 3).

3.2.4. Catalase (CAT) activity

There was significant depletion (p<0.001) in catalase activity by 48% in paracetamol treated group (23.46 ± 2.0) compared to the untreated control (45.18 ± 3.87). Treatment with *Parmelia perlata* at both doses D1 (39.67 ± 2.87, p<0.01) and D2 (41.24 ± 2.89, p<0.001) elevated the catalase activity significantly by 36% and 45% respectively. *Parmelia*
*perlata* alone did not show any significant difference as compared to control group (Table 3).

### 3.2.5. Glutathione peroxidase (GPx) activity

Rats treated with paracetamol showed a significant reduction (p<0.001) in the activity of GPx by 39% (149.4 ± 15.52) when compared with control animals (244.6 ± 15.5). However, significant restoration by 23% and 32% of GPx activity was observed in rats treated with *Parmelia perlata* at dose D1 (205.9 ± 8.7, p<0.05) and D2 (228.0 ± 7.88, P<0.01) respectively. No significant difference was found in the only *Parmelia perlata* treated group compared to the control group (Table 3).

### 3.2.6. Effect of *Parmelia perlata* on hepatic membrane damage (LPO)

Acetaminophen treatment caused significant increase (14.91 ± 1.15, p<0.001) in the level of MDA formation by 104% as compared with the control group (7.29 ± 0.77). *Parmelia perlata* administration were significantly effective in lowering down this acetaminophen induced lipid peroxidation at dose D1 (10.99 ± 1.01, p<0.05) by 54% and D2 (8.95 ± 0.7, P<0.01) by 45%. Only *Parmelia perlata* group showed no significant change as compared with control animals (Table 3).

### 3.2.7. Elevated Level of serum toxicity markers (AST, ALT and LDH) as a result of acetaminophen exposure, restored by *Parmelia perlata*.

In the present study, acetaminophen caused steep rise in the level of liver toxicity markers viz, AST (61%, p<0.001), ALT (86%, p<0.001) and LDH (63%, p<0.001) as compared with control group. Treatment of rats with *Parmelia perlata* showed significant protection in bringing back these elevated levels of serum toxicity markers by 36% (p<0.01), 28% (p<0.05) and 30% (p<0.001) at dose D1 and 60% (p<0.001), 68% (p<0.01) and 53% (p<0.001) at dose D2 respectively as compared with the paracetamol treated group (Table 4).

### 3.2.8. Effect of *Parmelia perlata* treatment on hepatic TNF-α level

TNF-α levels in the hepatic tissue of the acetaminophen treated rats was significantly elevated (p<0.001) by 32% as compared with control group. *Parmelia perlata* administration showed a significant reduction in the hepatic TNF-α levels at both doses D1 (p<0.01) and D2 (p<0.001) by 21% and 30% respectively as compared with the acetaminophen treated group, whereas no significant change in the TNF-α levels were found in only D2 group as compared to control group (Figure 4).
3.2.9. Effect of *Parmelia perlata* administration on caspase 3, 7 and 9 activities

Figure 5 shows the expression of caspase 3, 7 and 9 in liver tissue of rats. Acetaminophen treated group shows a significant elevation (p<0.001) of caspase 3, 7 and 9 as compared to control group. This elevation in caspase activities is significantly depleted by *Parmelia perlata* administration. (Caspase 3, D1: p<0.05; D2: p<0.01. Caspase 7, D1: NS; D2: p<0.01 and Caspase 9, D1: NS; D2: p<0.01) Animals treated with only high dose of *Parmelia perlata* did not show any significant change as compared to control group.

3.2.10. Histopathology of liver tissue

Hematoxylin-eosin-stained liver sections displayed representative hepatocellular morphological changes. Liver section in the vehicle control group showed a normal lobular structure. Liver section in acetaminophen alone group showed large areas of centrilobular necrosis, vacuolar degeneration and inflammatory cell infiltration. Liver section of the animals treated with *Parmelia perlata* D1 showed necrosis with almost the same degree as in acetaminophen group whereas, liver sections of rats treated with D2 of *Parmelia perlata* group showed a significant alleviation of liver injury. Liver section in the only D2 treated group showed absence of necrosis and almost normal lobular structure (Figure 6).
Table 3: Oxidative stress markers of liver toxicity and modulation by *Parmelia perlata*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Acetaminophen</th>
<th><em>P. perlata</em> D1 + Acetaminophen</th>
<th><em>P. perlata</em> D2 + Acetaminophen</th>
<th>Only <em>P. perlata</em> D2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CYP 450</strong> (nmol/mg protein)</td>
<td>13.91 ± 0.83</td>
<td>28.64 ± 0.63</td>
<td>16.81 ± 0.81**</td>
<td>14.43 ± 0.53***</td>
<td>14.12 ± 0.51</td>
</tr>
<tr>
<td><strong>LPO</strong> (nmolMDA formed/g tissue)</td>
<td>7.29 ± 0.77</td>
<td>14.91 ± 1.37**</td>
<td>10.99 ± 1.01</td>
<td>8.95 ± 0.7**</td>
<td>8.04 ± 0.67</td>
</tr>
<tr>
<td><strong>Catalase</strong> (nmol H₂O₂ consumed/min/mg protein)</td>
<td>45.18 ± 3.87</td>
<td>23.46 ± 2.0**</td>
<td>39.67 ± 2.87***</td>
<td>43.93 ± 2.89***</td>
<td>43.58 ± 2.98</td>
</tr>
<tr>
<td><strong>Glutathione Peroxidase</strong> (nmol NADPH oxidized/min/mg protein)</td>
<td>244.6 ± 15.5</td>
<td>149.4 ± 15.52**</td>
<td>205.9 ± 8.7</td>
<td>228.0 ± 7.88**</td>
<td>242.2 ± 13.28</td>
</tr>
<tr>
<td><strong>Glutathione reductase</strong> (nmol NADPH oxidized/min/mg protein)</td>
<td>318.8 ± 7.65</td>
<td>218.5 ± 7.67**</td>
<td>269.0 ± 9.97**</td>
<td>304.8 ± 14.21***</td>
<td>323.0 ± 5.87</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.E., n = 6

*P < 0.001 compared with the corresponding value for control group.

*P <0.05, **P <0.01 and ***P <0.001 compared with the corresponding value for acetaminophen treated group.

*Parmelia perlata* D1: 100mg/kg b. wt.; *Parmelia perlata* D2: 200mg/kg b. wt.
Acetaminophen induced hepatic injury

Table 4: Elevation of Serum liver toxicity markers (AST, ALT and LDH) by acetaminophen and their restoration by *Parmelia perlata*.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Acetaminophen</th>
<th>P. perlata D1 + Acetaminophen</th>
<th>P. perlata D2 + Acetaminophen</th>
<th>Only P. perlata D2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aspartate aminotransferase (AST or SGOT) (IU/L)</strong></td>
<td>47.39 ± 3.44</td>
<td>76.5 ± 5.8a</td>
<td>59.4 ± 6.8**</td>
<td>48.2 ± 4.9***</td>
<td>46.3 ± 6.1</td>
</tr>
<tr>
<td><strong>Alanine aminotransferase (ALT or SGPT) (IU/L)</strong></td>
<td>10.36 ± 0.9</td>
<td>19.3 ± 2.3a</td>
<td>16.4 ± 2.6*</td>
<td>12.3 ± 1.6**</td>
<td>10.6 ± 1.1</td>
</tr>
<tr>
<td><strong>Lactate dehydrogenase (LDH) (nmol NADH oxidised/min/mg protein)</strong></td>
<td>165.04 ± 16.3</td>
<td>268.3 ± 23.5a</td>
<td>218.8 ± 21.2***</td>
<td>181.2 ± 19.4***</td>
<td>164.2 ± 17.3</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.E., n = 6  
*a* P < 0.001 compared with the corresponding value for control group.  
*P <0.05, **P <0.01 and ***P <0.001 compared with the corresponding value for acetaminophen treated group.  
*Parmelia perlata* D1: 100mg/kg b. wt.; *Parmelia perlata* D2: 200mg/kg b. wt.
Figure 4: Effect of *Parmelia perlata* treatment on caspase activities

Graph showing the effect of *Parmelia perlata* on acetaminophen induced caspase activities. Each value represents mean ± S.E., n = 6

### P < 0.001 compared with the corresponding value for control group.

NS: non-significant, *P <0.05, **P <0.01 and ***P <0.001 compared with the corresponding value for acetaminophen treated group.

*Parmelia perlata* D1: 100mg/kg b. wt.; *Parmelia perlata* D2: 200mg/kg b. wt.
Graph showing the induction of TNF-α by acetaminophen administration in liver tissues and its amelioration by Parmelia perlata treatment.

Each value represents mean ± S.E., n = 6

**P < 0.01 and ***P < 0.001 compared with the corresponding value for acetaminophen treated group.

###P < 0.001 compared with the corresponding value for control group.

*Parmelia perlata* D1: 100mg/kg b. wt.; *Parmelia perlata* D2: 200mg/kg b. wt.
Figure 6: Histopathology of the liver tissue treated with *Parmelia perlata* and acetaminophen.

Representative photomicrographs (magnification x 400) showing histopathological architecture. **A)** Control liver. **B)** Acetaminophen treated group. **C)** Acetaminophen + *P. perlata* 100mg/kg b. wt (D1). **D)** Acetaminophen + *P. perlata* 200mg/kg b. wt (D2). **E)** Only *P. perlata* 200mg/kg b. wt (400x).
3.3.  Study 3: Amelioration of acetaminophen induced liver toxicity by Betaine

3.3.1.  Effect of Betaine on hepatic microsomal Cytochrome P450

Acetaminophen treated group showed significant elevation (p<0.001) in microsomal cytochrome P450 content by 106% (28.64 ± 0.63) as compared with the control group (13.91 ± 0.83) activity. Treatment of rats with Betaine at both doses significantly restored the level of paracetamol metabolising enzyme (cytochrome P450) by 84% (D1: 17.02 ± 0.31, p<0.05) and 95% (D2: 15.39 ± 0.78, p<0.01) respectively as compared with acetaminophen treated group. Animals treated with only Betaine showed no change in the Cytochrome p450 level as compared with the control group (Table 5).

3.3.2.  Treatment with Betaine prevents depletion in the GSH level

Acetaminophen induced hepatotoxicity is associated with the depletion of GSH. The level of GSH was decreased significantly in paracetamol treated group by 50% (0.024 ± 0.003, p<0.001) as compared to respective control group (0.048 ± 0.01). Pretreatment with Betaine showed significant elevation of the depleted GSH level by 38% at dose D1 (0.042 ± 0.001; p<0.01) and 48% at dose D2 (0.047 ± 0.007; p<0.001) as compared to paracetamol treated group (Table 5). No significant changes in GSH level were observed in the group treated with only Betaine as compared to the particular control groups.

3.3.3.  Glutathione-S- transferase (GST) activity

A significant decline in the GST activity by 36% was also observed in paracetamol treated group (37.57 ± 2.31, p<0.001) compared to the control (59.03 ± 3.62) group. Treatment with Betaine at doses D1 elevates GST activity non-significantly by 11% (NS), whereas at dose D2, GST activity was significantly increased by 26% (P<0.01) as compared with the paracetamol treated group. Whereas no significant change in the GST activity was observed in animals treated with only Betaine as compared with control group (Table 5).

3.3.4.  Catalase (CAT) activity

There was significant depletion (p<0.001) in catalase activity by 40% in paracetamol treated group (23.64 ± 1.69) compared to the untreated control (39.24 ± 1.44). Treatment with Betaine at both doses D1 (32.8 ± 2.9, p<0.05) and D2 (37.64 ± 2.3, p<0.01) elevated the catalase activity significantly by 23% and 35% respectively. Betaine alone did not show any significant difference as compared to control group (Table 5).
3.3.5. Glutathione peroxidase (GPx) activity

Rats treated with paracetamol showed a significant reduction (p<0.001) in the activity of GPx by 43% (136.3 ± 7.8) when compared with control animals (238.8 ± 7.31). However, significant restoration by 13% and 20% in GPx activity was observed in rats treated with Betaine at dose D1 (168.1 ± 9.3, p<0.05) and D2 (184.8 ± 6.1, P<0.01) respectively. No significant difference was found in the only Betaine treated group compared to the control group (Table 5).

3.3.6. Effect of Betaine on hepatic membrane damage (LPO)

Acetaminophen treatment caused significant increase (p<0.001) in the level of MDA formation by 237% (27.98 ± 2.18) as compared with the control group (8.29 ± 0.97). Betaine administration were significantly effective in lowering down this acetaminophen induced lipid peroxidation at dose D1 (19.59 ± 1.3, p<0.01) by 101% and D2 (10.5 ± 1.09, P<0.001) by 210%. Only Betaine group showed no significant change as compared with control animals (Table 5).

3.3.7. Elevated Level of serum toxicity markers (AST, ALT and LDH) as a result of acetaminophen exposure, restored by Betaine.

Acetaminophen caused steep rise in the level of liver toxicity markers viz, AST (56%, p<0.001), ALT (86%, p<0.001) and LDH (63%, p<0.001) as compared with control group. Treatment of rats with Betaine showed significant protection in bringing back these elevated levels of serum toxicity markers by 49% (p<0.01), 70% (p<0.01) and 30% (p<0.001) at dose D1 and 52% (p<0.001), 95% (p<0.001) and 53% (p<0.001) at dose D2 respectively as compared with the paracetamol treated group (Table 6).

3.3.8. Effect of Betaine treatment on hepatic TNF-α level

TNF-α levels in the hepatic tissue of the acetaminophen treated rats was significantly elevated (p<0.001) as compared with control group. Betaine administration showed a significant reduction in the hepatic TNF-α levels at both doses D1 (p<0.01) and D2 (p<0.001) respectively as compared with the acetaminophen treated group, whereas no significant change in the TNF-α levels were found in only D2 group as compared to control group (Figure 7).
3.3.9. Effect of Betaine administration on caspase 3, 7 and 9 activities

Expression of Caspase 3, 7 and 9 was significantly (p<0.001) elevated in the acetaminophen treated group as compared to the control group, whereas betaine treatment showed a significant reduction in the caspase activities (p<0.001) as compared to the acetaminophen treated group (Figure 8).
Table 5: Oxidative stress and antioxidant parameters of liver toxicity and modulation by Betaine

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Acetaminophen</th>
<th>Betaine D1 + Acetaminophen</th>
<th>Betaine D2 + Acetaminophen</th>
<th>Only Betaine D2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP 450 (nmol/mg protein)</td>
<td>13.91 ± 0.83</td>
<td>28.64 ± 0.63</td>
<td>17.02 ± 0.31</td>
<td>15.39 ± 0.78</td>
<td>14.1 ± 0.45</td>
</tr>
<tr>
<td>LPO (nmolMDA formed/g tissue)</td>
<td>8.29 ± 0.97</td>
<td>27.98 ± 2.18</td>
<td>19.59 ± 1.30</td>
<td>10.5 ± 1.09</td>
<td>11.65 ± 1.58</td>
</tr>
<tr>
<td>Catalase (nmol H₂O₂ consumed/min/mg protein)</td>
<td>39.24 ± 1.44</td>
<td>23.64 ± 1.69</td>
<td>32.8 ± 2.9</td>
<td>37.64 ± 2.3</td>
<td>38.57 ± 2.2</td>
</tr>
<tr>
<td>Glutathione Peroxidase (nmol NADPH oxidized/min/mg protein)</td>
<td>238.8 ± 7.31</td>
<td>136.3 ± 7.8</td>
<td>168.1 ± 9.3</td>
<td>184.8 ± 6.1</td>
<td>236.7 ± 6.84</td>
</tr>
<tr>
<td>Glutathione reductase (nmol NADPH oxidized/min/mg protein)</td>
<td>318.8 ± 7.7</td>
<td>218.5 ± 7.67</td>
<td>273.6 ± 7.8</td>
<td>313.5± 13.08</td>
<td>314.9 ± 10.42</td>
</tr>
<tr>
<td>Reduced Glutathione (µmol GSH conjugate/g tissue) (+: SD)</td>
<td>0.048 ± 0.01</td>
<td>0.024 ± 0.003</td>
<td>0.042 ± 0.001</td>
<td>0.047± 0.007</td>
<td>0.047 ± 0.01</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.E., n = 6

a P < 0.001 , b P<0.01 compared with the corresponding value for control group.

*P <0.05, **P <0.01 and ***P <0.001 compared with the corresponding value for acetaminophen treated group.
Table 6: Modulation of liver cytotoxicity serum markers by Betaine

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Acetaminophen</th>
<th>Betaine D1 + Acetaminophen</th>
<th>Betaine D2 + Acetaminophen</th>
<th>Only Betaine D2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aspartate aminotransferase (AST or SGOT) (IU/L)</strong></td>
<td>47.39 ± 3.44</td>
<td>73.89 ± 3.3\textsuperscript{a}</td>
<td>50.87 ± 3.2\textsuperscript{**}</td>
<td>49.47 ± 4.5\textsuperscript{**}</td>
<td>45.78 ± 3.6</td>
</tr>
<tr>
<td><strong>Alanine aminotransferase (ALT or SGPT) (IU/L)</strong></td>
<td>10.36 ± 0.9</td>
<td>19.3 ± 2.3\textsuperscript{b}</td>
<td>12.08 ± 1.7\textsuperscript{*}</td>
<td>9.5 ± 1.5\textsuperscript{**}</td>
<td>9.13 ± 0.98</td>
</tr>
<tr>
<td><strong>Lactate dehydrogenase (LDH) (nmol NADH oxidised/min/mg protein)</strong></td>
<td>165.04 ± 16.3</td>
<td>268.3 ± 23.5\textsuperscript{a}</td>
<td>218.4 ± 18.3\textsuperscript{***}</td>
<td>182.3 ± 17.3\textsuperscript{***}</td>
<td>164.1 ± 16.5</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.E., n = 6
\( a \ P < 0.001 \), \( b \ P<0.01\) compared with the corresponding value for control group.
\( *P <0.05, **P <0.01 \) and \( ***P <0.001 \) compared with the corresponding value for acetaminophen treated group.
Betaine D1: 50mg/kg b. wt
Betaine D2: 100mg/kg b. wt
Figure 7: TNF-α in liver tissue of rats treated with acetaminophen and its modulation by betaine administration

Each value represents mean ± S.E., n = 6

### P < 0.001 compared with the corresponding value for control group.

*P <0.05, **P <0.01 and ***P <0.001 compared with the corresponding value for acetaminophen treated group. Betaine D1: 50mg/kg b. wt; Betaine D2: 100mg/kg b. wt

Figure 8: Effect of betaine on acetaminophen induced apoptosis markers.

Each value represents mean ± S.E., n = 6

### P < 0.001 compared with the corresponding value for control group.

*P <0.05, **P <0.01 and ***P <0.001 compared with the corresponding value for acetaminophen treated group. Betaine D1: 50mg/kg b. wt; Betaine D2: 100mg/kg b. wt
4. Discussion

Liver is the primary target for drug induced toxicity, because of its role in clearing and metabolizing majority of chemicals and drugs through the process called detoxification. Drug induced liver disorders occurred frequently can be life threatening and mimic all forms of liver diseases (Banskota et al., 2000; DeFeuids et al., 2003; Takeoka and Dao, 2003).

In the present study, betaine and methanolic extracts of *Carum carvi* and *Parmelia perlata* was observed to exhibit hepatoprotective effect as demonstrated by a significant decrease in liver function markers, also cellular antioxidant defence and by preventing liver histopathological changes in rats induced with hepatotoxicity.

It has been reported that depletion of GSH is associated with the acetaminophen toxicity (Jollow et al., 1973). In over doses, acetaminophen is metabolised by cytochrome P450 to highly reactive NAPQI, which alkylates and oxidises the intracellular GSH, which results in the depletion of GSH content (Savides and Oehne, 1983). This reduction in the levels of GSH subsequently lead to the depletion in the cellular defence armoury (antioxidants) simultaneously resulting in the generation of oxidants within the cells. Altered oxidant and antioxidant levels within the cells lead to lipid peroxidation of the membrane lipids by abstracting hydrogen from a polyunsaturated fatty acid and, thereby disrupting mitochondrial and possibly nuclear function, leading to oxidative stress (Handa and Sharma, 1990; Sabira and Rocha, 2008). Our results are also in accordance with the above facts that, acetaminophen administration to rats suppresses the cytochrome P450 in the liver tissue with the subsequent depletion of the GSH levels. In the present study we have also observed that the treatment with *Carum carvi*, *Parmelia perlata* and betaine normalizes the depleted level of cytochrome P450. We have also found that GSH depletion- the prime marker of acetaminophen toxicity, is also elevated in the animals treated with *Carum carvi*, *Parmelia perlata* and betaine. Moreover, these modulators also protect the cells against the peroxidative effects of acetaminophen induced peroxidation of membrane lipids.

In fact, cells have a number of mechanisms to protect themselves from the toxic effects of free radicals generated by acetaminophen and other toxicants. Superoxide dismutase removes superoxide (O2) by converting it to H2O2, which can be rapidly converted to water by catalase and glutathione peroxide (GPx) (Halliwell et al., 1992). In addition, a large reserve of reduced glutathione is present in hepatocytes for detoxification of
xenobiotics or free radicals. However, oxidative stress results in toxicity when the rate at which the free radicals are generated exceeds the cell’s capacity for their removal. This enhanced oxidative stress in the cells due to the interaction of free radicals with macromolecules results in the increased activities of Xanthine oxidase (an important non-CYP oxidative enzyme) which is involved in the metabolism of acetaminophen (Hinson et al., 2002). Increased activities of Xanthine oxidase can be used as an important tool to determine drug induced liver toxicity (Tirmenstein and Nelson, 1990; Rashidi and Nazemiyeh, 2010). Our results confirm the above facts of acetaminophen toxicity and contrarily, administration of Carum carvi, Parmelia perlata and betaine to rats restores the antioxidant enzymes to the normal levels confirming their potent hepatoprotective efficacy against acetaminophen induced liver damage.

Furthermore, Oxidative stress caused by acetaminophen results in the release of LDH, a marker of cell damage, and the release of liver toxicity enzymes (ALT and AST) (Shireen et al., 2008). The estimation of enzymes in the serum is a useful quantitative marker of the extent and type of hepatocellular damage. The rats treated with an overdose of acetaminophen developed significant hepatic damage, which was observed by a substantial increase in the concentration of serum enzymes (AST, ALT and LDH). Administration of Carum carvi, Parmelia perlata and betaine treatment resulted in a significant reduction of acetaminophen induced elevation of AST, ALT and LDH levels and appears to be protective in reducing the injurious effect of acetaminophen.

Several studies have reported that inflammatory cytokines are increased in acetaminophen toxicity. Blazka reported the up-regulation of TNF-α in the acetaminophen treated mouse (Blazka et al., 1995a; Blazka et al., 1996). In addition, he showed that selective inhibition of TNF-α partially decreased toxicity for a period of time. Consistent with these findings, we have also found the acetaminophen induced up-regulation of TNF-α as compared to the untreated control animals and treatment of rats with Carum carvi, Parmelia perlata and betaine significantly down regulates the acetaminophen induce elevation in TNF-α levels. It has been demonstrated by Kazuyoshi and Kass et al., that apoptosis plays a critical role in acetaminophen induced liver injury (Kass et al., 2003; Kazuyoshi et al., 2004). They also concluded that inhibition of apoptosis can be a better tool in the prevention of acetaminophen induced liver damage. In the present study, we have evaluated the activities of effector caspases (Caspases 3 and 7) and initiator caspases (Caspase 9). Caspase-3 and 7 are one of the key executioners of apoptosis, capable of cleaving or degrading many key
proteins such as nuclear lamins, fodrin, and the nuclear enzyme poly (ADP-ribose) polymerase (PARP). Our results showed that there is the significant induction of Caspase activity in the acetaminophen treated animals as compared to the control animals. These increased activities of caspases were significantly reduced in the animals treated with *Carum carvi*, *Parmelia perlata* and betaine. This reduction in the activities of caspases by the treatment of these modulators implies the reduction of acetaminophen induced apoptosis, so as the liver toxicity.

The results of the above biochemical enzymatic activities, oxidative stress markers, serum toxicity markers are in complete conformity with the histopathological alterations observed in the acetaminophen treated animals and concomitant normalization of the hepatic architecture by the treatment with *Carum carvi*, *Parmelia perlata* and betaine administrations. In conclusion, our results confirm that the modulators of the present study (*Carum carvi*, *Parmelia perlata* and betaine) were potent antioxidants and hepatoprotective agents and can be used against acetaminophen induced liver injury. Further, it can be concluded that treatment with a *Carum carvi*, *Parmelia perlata* and betaine reduced hepatic damage in Wistar rats induced by the administration of the toxic dose of acetaminophen. Supplementation of rats with the above modulators reduced the pathological and histological changes in the liver, as well as prevented acetaminophen induced elevation in oxidative stress, up-regulation of pro-inflammatory cytokines and apoptosis. However, further pharmacokinetic and pharmacodynamic studies are required to commence that these drugs to go for clinical trials.