9. HEPATOPROTECTIVE ACTIVITY OF HYALURONIC ACID AGAINST ACETAMINOPHEN INDUCED TOXICITY IN MALE WISTAR RATS

9.1. Introduction

Liver is an important target of the toxic drugs, toxins, xenobiotics and oxidative products. It is the main organ involved in the metabolism of biological toxins and medicinal agents. Such metabolism is always associated with the disturbance of hepatocyte biochemistry and generation of ROS (Fernandez-Checa and Kaplowitz, 2005). Drug/chemicals-induced liver injury has become a major public health problem, in that various pharmacological or chemical substances are known to cause hepatic disorders such as acetaminophen, alcohol, carbon tetrachloride, D-galactosamine and dimethyl nitrosamine (Bissel et al., 2001; Lee, 2003). Therefore, a potential novel approach is needed to develop antioxidant drugs to treat and protect liver injury and liver disease (Bansal et al., 2005).

Acetaminophen (APAP) is one of the best-selling analgesics in the US and it is the most popular drugs in the world (Kaufman et al., 2002). Although it is safe in therapeutic doses, overdose of APAP can cause severe liver injury. The first reports of APAP hepatotoxicity in humans appeared in the literature during 1960s (Davidson and Eastham, 1966). APAP overdose has become the most common cause of acute liver failure in most Western countries. In the US alone, misuse of APAP is responsible for about 56,000 emergency room visits, 26,000 hospitalizations, and nearly 500 deaths every year (McGill et al., 2012).

The majority of a therapeutic dose (>90%) of APAP is glucuronidated or sulfated and then excreted. A small percentage is metabolized by cytochrome P450 enzymes (CYP) to the reactive intermediate N-acetyl-p-benzoquinone imine
(NAPQI), which is readily detoxified by conjugation with glutathione (GSH) (Nelson, 1990). The hepatotoxicity of acetaminophen is believed to be mediated by the reactive metabolite N-acetyl-p-benzoquinone imine. However, the mechanism by which this metabolite produces the toxicity is unknown. The metabolite, which is both an electrophile and an oxidizing agent, may covalently bind to critical proteins, or it may initiate oxidative damage (Gibson et al., 1996).

Acetaminophen induced oxidative stress results in lipoperoxidation, protein thioloxidation, mitochondrial endoplasmic reticulum injury, altered homeotaxis and irreversible DNA damage characterized by protein adduct formation (Sies, 1993). Several antioxidant systems occur in body which includes super oxide dismutase, glutathione peroxidase, catalase, vitamin C and vitamin E (Sies, 1993; Liu et al., 1999). The production of reactive oxygen species is a well known physiological process. The free radical induced oxidative damage of cell membranes, DNA and protein might be the cause of several diseases (Nishigori, 2006). Superoxide dismutase, catalase and glutathione peroxidase a tripeptide constitute mutually a supportive team of defense against reactive oxygen species (Prabu et al., 2012). They play a critical role as a marker of chemoprevention due to their antioxidant and detoxification properties.

The processes of liver repair and of fibrogenesis resemble to the wound healing process. When injury and the associated acute inflammation response result in moderate cell necrosis and extra cellular matrix damage, tissue repair normally takes place. In this process, dead cells are replaced by normal tissue, with regeneration of specialized cells by proliferation of the surviving ones, formation of granulation tissue and tissue remodeling with scar formation. The specific regenerative capacities of the liver generally allow it to reconstitute itself entirely
following acute, moderate lesions (Bataller and Brenner, 2005). Antioxidants are able to reduce hepatic inflammation and fibrosis, thus slowing or even preventing progression to cirrhosis.

The rationale behind this, the isolated HA of *M. casta* *in vivo* assessment was based on the percentage of yield as well as purity level. Besides, it shows higher potential of *in vitro* antioxidant activity of *M. casta* HA when compared to the *A. pleuronetus*.

Hence, the present study evaluated the effect of isolated HA (*M. casta*) on APAP induced hepatotoxicity in male Wistar rats by analyzing the level of lipid peroxidation, enzymatic and non-enzymatic antioxidants, liver marker enzymes and lipid profile. The histopathology of liver tissues was also examined.

9.2. Materials and Methods

9.2.1. Animal model

Male Wistar rats (weighing 100-150 g) were housed in well ventilated rooms (temperature: 23 ± 2 °C, humidity 65-70% and 12 h light/dark cycles) at Central Animal House, Department of Experimental Medicine RMMCH, Annamalai University and fed with standard pellet diet and water *ad libitum*. All studies were carried out in accordance with Indian National law of animal care and use and committee (Ethical approval number 1065 and date 29.11.2013) for the purpose of control and supervision of animals at RMMCH, Annamalai University, Annamalai Nagar.
9.2.2. Experimental design

Male Wistar rats weighing between 100-150 g were kept under standard laboratory conditions for one week before the commencement of experiments. The rats were randomly divided into four groups of six rats each. Then, the animal was treated as follows:

**Group 1** : Normal control rats fed with standard diet and pure drinking water for 15 days.

**Group 2** : Rats induced with acetaminophen (500 mg/kg b.w.) through i.p. on 15\textsuperscript{th} day only.

**Group 3** : Rats treated with hyaluronic acid from *M. casta* (80 mg/kg b.w orally) alone for 14 days.

**Group 4** : Rats treated with hyaluronic acid from *M. casta* (80 mg/kg b.w orally) for 14 days and treated with acetaminophen (500 mg/kg b.w) through i.p.

The blood and liver samples were collected and the following parameters were employed.

9.2.3. Estimation of lipid peroxidation

9.2.3.1. Estimation of Thiobarbituric acid reactive substances (TBARS)

The levels of thiobarbituric acid reactive substances (TBARS) in liver tissue and serum were estimated by the method of Yagi (1987). Standard malondialdehyde (MDA) solution (1-5 nmol) in 4.0 ml volume and blank containing 4.0 ml distilled water were processed along with the test samples. The values were expressed as mM/100 g wet tissue and nM/ml serum.
9.2.3.2. Estimation of lipid hydroperoxidation

Lipid hydroperoxides in tissue were estimated by the method of Jiang et al. (1992). In this method, oxidation of ferrous ions (Fe^{2+}) under acidic conditions in the presence of xylenol orange leads to the formation of a chromophore with an absorbance maximum at 560 nm. The values were expressed as mmol/100 g wet tissue and mM/dl serum.

9.2.4. Assay of enzymatic antioxidants

9.2.4.1. Assay of superoxide dismutase (SOD)

The level of SOD in the liver and serum was determined according to the procedure of Kakkar et al. (1984). Superoxide radicals react with nitrobluetetrazolium (NBT) in the presence of NADH and produce formazan blue. SOD removes the superoxide radicals and inhibits the formation of formazan blue. The intensity of colour is inversely proportional to the level of the enzyme.

One unit of the enzyme activity was defined as the enzyme reaction which gave 50% inhibition of NBT reduction in one minute under the assay conditions and expressed as specific level in units/mg protein.

9.2.4.2. Assay of Catalase (CAT)

The level of catalase in the liver and serum was assayed by the method of Sinha (1972). Dichromate in acetic acid was converted to perchromic acid and then to chromic acetate, when heated in the presence of H₂O₂. The chromic acetate formed was measured at 620 nm. The level of catalase was expressed as μmoles of H₂O₂ consumed/min/mg of protein.
9.2.4.3. Assay of Glutathione peroxidase (GPx)

The presence of Glutathione peroxidase in the serum and liver was assayed by the method of Rotruck et al. (1973). A known amount of enzyme preparation was allowed to react with H$_2$O$_2$ in the presence of GSH for a specified time period. The remaining GSH was measured by the method of Ellman (1959). The level of GPx was expressed as µg of GSH consumed/min/mg protein.

9.2.5. Assay of non-enzymatic antioxidants

9.2.5.1. Estimation of reduced glutathione (GSH)

GSH was estimated by the method of Ellman (1959). This method was based on the development of yellow colour when 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) was added to the compounds containing sulphhydryl groups. The amount of glutathione was expressed as mg/dl serum and mmol/g wet tissue.

9.2.6. Estimation of marker enzymes

9.2.6.1. Activity of aspartate transaminases (AST)

The level of serum and tissue aspartate transaminases was assayed using the Beacon-Liquizyme (Code no.S11 C) kit by U.V. Kinetic method. The values were expressed as IU/L.

9.2.6.2. Activity of alanine transaminases (ALT)

The level of serum and tissue alanine transaminases was assayed using the Beacon-Liquizyme (Code no.S11 C) kit by U.V. Kinetic method. The values were expressed as IU/L.
9.2.7. Estimation of lipid profile

9.2.7.1. Extraction of lipids

Lipid was extracted from liver tissue by the method of Folch et al. (1957) using chloroform-methanol mixture (CHCl₃: CH₃OH) (2:1 v/v) and quantified gravimetrically. The dried residue of lipid was dissolved in 5 ml of CHCl₃: CH₃OH mixture (2:1 v/v) and transferred into a centrifuge tube. 2 ml of 0.1 M potassium chloride was added, shaken well and centrifuged. The upper aqueous layer containing gangliosides was discarded. Then the CHCl₃- CH₃OH -potassium chloride mixture (1:10:10 v/v) was added to the precipitate and centrifuged. This washing was repeated thrice and each time, the upper layer was discarded. The lower layer was made up to 5 ml and used for the analysis of cholesterol, HDL cholesterol, triglycerides and free fatty acids. The serum was also treated as above for the estimation of lipids.

9.2.7.2. Estimation of triglycerides

Triglycerides were quantified using the Beacon-Liquizyme (Code no.S13 C) kit by GPO/POD method. The triglycerides were expressed as mg/dl of serum and mg/g in wet tissue.

9.2.7.3. Estimation of cholesterol

Cholesterol was estimated using the Beacon-Liquizyme (Code no.S06) kit by CHOD/POD method. The cholesterol was expressed as mg/dl of serum and mg/g in wet tissue.

9.2.7.4. Estimation of HDL cholesterol

HDL cholesterol was estimated using the Beacon-Liquizyme (Code no.S06) kit by CHOD/POD method. The HDL cholesterol was expressed as mg/dl of serum and mg/g in wet tissue.
9.2.7.5. Estimation of Free Fatty Acids

Free fatty acids in the serum and tissue were estimated by the method of Falholt et al. (1973). Free fatty acids were extracted with chloroform-heptane-methanol mixture to eliminate interference from phospholipids. The extract was shaken with a high density copper reagent at pH 8.1. The copper soaps remained in the upper organic layer from which an aliquot was removed and copper content was determined calorimetrically by treating with diphenylcarbazide. The free fatty acids were expressed as mg/dl of serum and mg/g in wet tissue.

9.2.8. Histopathology

In the case of histopathological examination, the liver was dissected out from one rat in each group of experimental rats after cervical decapitation. The liver tissue was fixed with 10% formalin. After proper dehydration of the tissue, it was embedded in paraffin wax. The sections (6 μ thickness) were made using a rotary microtome and stained with hematoxylin and eosin (Pantin, 1962). The sections were observed under motictrinocular stereo zoom microscope (DMWBI series) and photographs were taken.

9.3. Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) using SPSS software 16.0 followed by Duncan’s multiple range test (DMRT). Results were expressed as mean ± S.D. from six rats in each group. \( P \) values <0.05 were considered as significant.
9.3. Results

9.3.1. Estimation of lipid peroxidation

9.3.1.1 Estimation of thiobarbituric acid reactive substances (TBARS)

There was an elevation in the level of TBARS in serum and liver in APAP
induced group, whereas there were no significant change in TBARS level in HA
alone treated rats when compared to normal. In the case of isolated HA, there was
a significant decrease in the levels of TBARS when compared to inducer group.
Table 4 and Fig. 23 and 24 show the level of TBARS in serum and tissue.

Table 4. Changes in the levels of TBARS and lipid hydroperoxides in serum
and tissues (Values are mean ± S.D from 6 rats in each group).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum (mM/100 ml)</th>
<th>Liver (mM/100 g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>4.8 ± 0.11a</td>
<td>12.16 ± 0.12a</td>
</tr>
<tr>
<td>Group II</td>
<td>7.4 ± 0.17a</td>
<td>15.79 ± 0.15b</td>
</tr>
<tr>
<td>Group III</td>
<td>4.62 ± 0.14a</td>
<td>12.03 ± 0.22a</td>
</tr>
<tr>
<td>Group IV</td>
<td>5.38 ± 0.07c</td>
<td>13.65 ± 0.13c</td>
</tr>
</tbody>
</table>

abc p< 0.05 the values not sharing a common superscript letter are significantly
different. a, b & c comparisons were made between groups 1, 2, 3 & 4.

9.3.1.2 Estimation of lipid hydroperoxides

The levels of tissue and serum hydroperoxides of APAP induced group
showed a significant increase when compared to the normal group. The level was
decreased significantly in animals treated with HA along with APAP. Group III
rats treated with HA alone showed the same level of activity when compared with
normal group (Table 5 and Figs. 23 & 24).
Table 5. Changes in the levels of lipid hydroperoxides in serum and tissues
(Values are mean ± S.D from 6 rats in each group).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum (mM/100 ml)</th>
<th>Liver (mM/100 g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>3.12 ± 0.93 a</td>
<td>10.71 ± 0.97 a</td>
</tr>
<tr>
<td>Group II</td>
<td>6.75 ± 0.94 b</td>
<td>14.63 ± 0.97 b</td>
</tr>
<tr>
<td>Group III</td>
<td>3.43 ± 0.92 a</td>
<td>10.58 ± 0.92 a</td>
</tr>
<tr>
<td>Group IV</td>
<td>5.30 ± 0.98 c</td>
<td>12.89 ± 0.92 c</td>
</tr>
</tbody>
</table>

\(^{a,b,c} p < 0.05\) the values not sharing a common superscript letter are significantly different. \(^{a,b,c}\) comparisons were made between groups 1, 2, 3 & 4.

![Graph showing changes in TBARS and lipid hydroperoxides in liver](image)

**Fig. 23.** Level of TBARS and lipid hydroperoxides in liver.
9.3.2 Assay of antioxidant enzymes

The changes in the activity of superoxide dismutase, catalase and glutathione peroxidase are given in Table.6 and Figs. 25 & 26. The activities of SOD, CAT and GPx were found decreased significantly in APAP induced group when compared to normal. But the level of antioxidant enzymes in rats treated with HA alone was same to the control group. In group IV rats treated with HA and APAP showed a significant increase in the activities of SOD, CAT and GPx in liver and serum, when compared to APAP induced group.
Table 6. Changes in the activity of superoxide dismutase, catalase and glutathione peroxidase in Serum and Tissues (Values are mean ± S.D from 6 rats in each group)

| Groups | Superoxide dismutase | | Catalase | | Glutathione peroxidase | |
|--------|----------------------|------------------|------------------|------------------|------------------|
|        | Liver (U/mg protein) | Serum (U/ml) | Liver (U/mg protein) | Serum (U/ml) | Liver (U/mg protein) | Serum (U/ml) |
| Group I | 13.58 ± 0.22 a | 19.76 ± 0.17 a | 48.35 ± 2.86 a | 56.58 ± 2.23 a | 42.47 ± 0.34 a | 46.03 ± 0.26 a |
| Group II | 8.76 ± 0.33 b | 12.48 ± 0.22 b | 33.74 ± 1.64 b | 41.63 ± 1.74 b | 35.86 ± 0.67 b | 32.69 ± 0.45 b |
| Group III | 13.63 ± 0.26 c | 19.82 ± 0.20 c | 48.57 ± 1.50 c | 57.02 ± 1.17 c | 42.56 ± 0.39 c | 46.31 ± 0.30 c |
| Group IV | 11.29 ± 0.23 c | 16.71 ± 0.13 c | 39.68 ± 1.60 c | 52.48 ± 1.2 c | 40.18 ± 0.45 c | 43.78 ± 0.34 c |

\[^{a,b,c}p< 0.05\] the values not sharing a common superscript letter are significantly different. \[^{a,b,c}\] comparisons were made between groups 1, 2, 3 & 4.

![Activity of SOD, CAT & GPx in liver tissue](image)

**Fig 25. Activity of SOD, CAT & GPx in liver tissue.**
9.3.3 Assay of non-enzymatic antioxidants

9.3.3.1 Estimation of reduced glutathione

The level of reduced glutathione was given in Table 7 and Figs. 27 & 28. The GSH showed a significant decrease in liver and serum of APAP induced group when compared to normal. On treatment with HA along with APAP, there was a significant increase in the levels of GSH which showed positive activity towards normal level. Rats treated with HA alone showed the significant level of reduced glutathione in both serum and tissue when compared with control group.
Table 7. Changes in the levels of reduced glutathione in serum and tissues
(Values are mean ± S.D from 6 rats in each group)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum (mg/dl)</th>
<th>Liver (mM/g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>12.78 ± 0.43</td>
<td>10.06 ± 0.32</td>
</tr>
<tr>
<td>Group II</td>
<td>7.59 ± 0.30</td>
<td>6.32 ± 0.29</td>
</tr>
<tr>
<td>Group III</td>
<td>12.85 ± 0.72</td>
<td>10.33 ± 0.59</td>
</tr>
<tr>
<td>Group IV</td>
<td>11.46 ± 0.43</td>
<td>8.54 ± 0.54</td>
</tr>
</tbody>
</table>

\(^{a-c}p < 0.05\) the values not sharing a common superscript letter are significantly different. \(^{a, b, c}\) comparisons were made between groups 1, 2, 3 and 4.

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Fig.27. Level of GSH in liver
9.3.4. Estimation of liver marker enzymes

9.3.4.1 Activities of Alanine transaminases (ALT) and Aspartate transaminases (AST)

Activities of ALT and AST are shown in Table.8 and Figs. 29 & 30. The activities of ALT and AST in serum and tissue were observed as increasing in APAP induced group when compared to that of the normal treated group. Treatment with HA along with APAP illustrated decreased activities of ALT and AST compared to APAP induced group that showed positive activity towards the normal. Group III rats treated with HA alone showed no significant difference when compared with normal group I rats.
Table 8. Changes in the activity of liver marker enzymes in serum and tissue

(Values are mean ± S.D from 6 rats in each group)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Alanine transaminase (IU/L)</th>
<th>Aspartate transaminase (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>Tissue</td>
</tr>
<tr>
<td>Normal</td>
<td>83.52 ± 2.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>126.93 ± 1.98&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>APAP</td>
<td>157.16 ± 4.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>216.53 ± 4.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HA</td>
<td>83.97 ± 1.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>126.53 ± 2.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>APAP + HA</td>
<td>96.21 ± 3.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>149.88 ± 2.92&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup>p < 0.05 the values not sharing a common superscript letter are significantly different. <sup>a,b,c</sup>comparisons were made between groups 1, 2 & 3.

Fig.29. Level of marker enzymes in liver.
9.3.5 Estimation of lipid profile

The levels of serum and tissue cholesterol, triglycerides and FFA were significantly increased in APAP induced group when compared to that of the normal. There was a marked decrease in the level of cholesterol, triglycerides and FFA in APAP and HA treated group when compared to APAP alone induced group. Whereas, the level of HDL cholesterol in serum and tissue were significantly decreased in APAP induced group when compared to that of normal. Treatment with HA along with APAP increased the level of HDL cholesterol compared to APAP group. The level of lipids in HA alone treated group showed no significant difference when compared with normal (Tables. 9 and Figs. 31 & 32).
Fig. 31. Level of lipid profile in serum.

Fig. 32. Level of lipid profile in liver tissue.
9.3.6 Histopathology

The histopathological effect on the liver cells was examined. The sections from the liver of control group illustrates normal control liver, portal areas were clear, the hepatic lobular architecture was normal, whereas the sections from APAP treated liver shows damaged hepatocytes, the surface of liver was unsmooth, lobular structure was damaged.

Fig.33. Histopathological sections of liver (a) control rats; (b) APAP treated rats; (c) HA alone treated rats; (d) HA + APAP treated rats.
9.4 DISCUSSION

In the present investigation, the hepatoprotective effect of HA against APAP induced hepatic injury in male Wistar rat was analyzed. Many chemical agents such as CCl₄, APAP, thioacetamide and polycyclic aromatic hydrocarbons cause liver damage in humans and animals (Siegers et al., 1988). APAP induced hepatic damage is the second leading cause of liver transplantation and accounts for considerable levels of morbidity and mortality. Thus, the protective mechanisms of the liver are of special concern. Several natural antioxidants reduce oxidative stress and protect from hepatic damage. Oxidative stress is also considered to be involved in the induction of hepatotoxicity by APAP. The one electron oxidation of APAP by cytochrome P450 (CYP) may generate reactive oxygen species (ROS). Hydrogen peroxide and superoxide are produced during metabolic activation of APAP in the mixed function oxidase system (Nordblom and Coon, 1977; Kuthan et al., 1978; De Vries, 1981).

Initially, Malondialdehyde (MDA) levels in the liver and serum was evaluated because MDA is a main marker of endogenous lipid peroxidation, the increase of MDA indicates that peroxidative damage increased. Likewise, the present study, APAP treated group showed a significantly increased level of TBARS and LOOH in serum and tissue. But, the level was decreased significantly in the animals treated with HA along with APAP. It shows the protective effect of HA and this effect coincides with the result of Ke et al. (2011) in which low molecular weight HA showed inhibiting the ability for lipid peroxidation in the reduction of MDA production in immunosuppressed mice. Trommer et al. (2003) also reported that different molecular size, weight of HA showed nearly similar effects on the level of generated lipid peroxidation products in TBARS.
Biological systems protect themselves against the damaging effects of activated species by several means. These include free radical scavengers and chain terminators; enzymes such as SOD, CAT and GPx system (Kurata et al., 1993). The major antioxidant enzymes, including SOD, CAT and GPx are regarded as the first line of the antioxidant defense system against ROS generated in vivo during oxidative stress. GPx is an important enzyme in protecting against chemically caused cytotoxicity which can eliminate the reactive intermediates by conjugation and hydroperoxide reduction or scavenge free radicals by direct quenching. SOD dismutases superoxide radicals to form hydrogen peroxide, which in turn is decomposed to water and oxygen by GPx and CAT, thereby preventing the formation of hydroxyl radicals (Cui et al., 2010). Therefore, these enzymes act collectively at different sites in the metabolic pathway of free radicals. In the present study, there was a significant decrease of SOD activity in APAP treated rats. The observed increase of SOD activity in the HA and APAP treated rats suggests that the HA has an efficient protective mechanism in response to ROS. Further, these findings also indicated that HA may be associated with decreased oxidative stress and free radical-mediated tissue damage. Cyclophosphamide induced cytotoxicity may occur by either necrosis or apoptosis, the activity of SOD, CAT and GPx of both livers and serum decreased markedly when the animals were treated with cyclophosphamide. The enhanced activity of SOD, CAT and increased GPx were observed in animals treated with both cyclophosphamide and low molecular weight HA (Ke et al., 2011). In the present investigation, it was observed that significant reduction in GSH levels in serum and liver in APAP treated rats. The increase in hepatic and serum GSH levels in the rats treated with
HA along inducer APAP may be due to de novo GSH synthesis or GSH regeneration. It was confirmed from the present study that the HA was dose dependent and significantly restored hepatic GSH content towards normal in APAP intoxicated rats indicating decreased free NAPQI level in the blood.

Reduced glutathione (GSH) constitutes the first line of defense against free radicals (Recknagel et al., 1989). Several diseases have been associated with changes in GSH levels and reduce the resistance to oxidative stress. The level of GSH and the activities of GPx were used to monitor the balance of oxidative stress and the chemopreventive ability (Hatono et al., 1996). Regarding non-enzymatic antioxidants, GSH is a critical determinant of tissue susceptibility to oxidative damage and the depletion of hepatic GSH has been shown to be associated with an enhanced toxicity of chemicals (Hewawasam et al., 2003). Hence the present study confirmed that the effect of isolated HA in APAP induced hepatotoxicity in Wistar rat by biochemical changes of TBARS, enzymatic activities and non enzymatic activities. In the same way, Ross et al. (2012) also reported that the seaweed polysaccharides demonstrated efficient hepatoprotective effect on APAP induced liver toxicity.

The estimation of enzymes in the serum is a useful quantitative marker of the extent and the type of hepatocellular damage (Jain and Singhai, 2011). Liver transaminases such as AST (aspartate transaminase) or SGOT (serum glutamic oxaloacetic transaminase), and ALT (alanine transaminase) or SGPT (serum glutamic pyruvic transaminase) have still remained the gold standards for the assessment of liver injury, and have been used as biomarkers of choice for decades (Howell et al., 2014). In the present investigation, analysis of ALT and AST shows
the extent of liver injury on APAP treatment. A striking elevation in the activity of AST in both serum and tissues was observed in APAP induced group. Likewise, ALT level also increased in the same group. This increased level of serum ALT and AST in mice treated with APAP indicate a deterioration of the hepatic functions due to the toxic effects of the drug. The increase in collagen contents implicates oxidative liver injury while the histopathological data of the tissues confirm the APAP induced organ damage (Toklu et al., 2006). High levels of AST indicate liver damage, such as that due to viral hepatitis as well as cardiac infarctions and muscle injury. ALT is more specific to the liver and thus serves as better parameters for detecting liver injury. Hence in the present finding, the HA along with the APAP treated group indicates a striking decreases in activity of ALT and AST when compared to Group II. Also, the HA treated group were relatively unchanged when compared with the control group which implies the protective effect of HA. This was related to the Campo et al. (2004) statement that the combinational treatment with HA and chondroitin sulphate was able to reduce the transaminase levels.

Free fatty acids from different sources can accumulate as triglycerides in the liver as a consequence of a variety of metabolic disturbances (Lieber, 1992). The fatty liver results mainly from the accumulation of triglycerides (Cunnane, 1987). Excessive accumulation of hepatic triglycerides termed hepatic steatosis, can be induced by APAP. The rate limiting step in triglyceride biosynthesis is the formation of lysophosphatidic acid from glycerol-3-phosphate catalyzed by GPAT (Dircks and Sul, 1997). Over expression of GPAT was resulted in accumulation of liver triglycerides (Linden et al., 2006). In the present study, APAP treatment
caused a significant increase in the level of FFA, TG and cholesterol when compared to control rats and treatment with HA reversed the effect of APAP treatment and brought the values to near normal. The observed increment of FFA may directly due to lipid breakdown and indirectly due to oxidation and its conversion of FFA. The increment FFA level can increase the synthesis of other major lipids and activate NADPH or NADH dependent microsomal peroxidation (Kaffarnik et al., 1978). On the other hand, the decrement in FFA content in HA treated APAP groups may lead to decreased synthesis of cholesterol, triglycerides and extent of lipid peroxidation. HDL cholesterol levels were significantly lower when compared to the normal. Supplementation with HA significantly prevented the APAP induced elevation of HDL cholesterol in serum and hepatic tissue. Likewise, the sulphated polysaccharide from the Sargassum polycystum crude extract showed the same effect on lipid metabolism against acetaminophen induced hyperlipidemia during toxic hepatitis (Raghavendran et al., 2005)

Hepatotoxicity induced by APAP was further confirmed by abnormal histological findings. The liver cells of control group illustrated the normal control liver with normal central vein and portal vein, the hepatic lobular architecture was normal and the liver sections of HA alone treated group was same that of control group, whereas the sections from APAP treated liver illustrates damaged hepatocytes, unsMOOTH surface of liver, lobular structures was damaged. The liver sections of rats treated with HA along with APAP showed a consistent preservation of hepatocyte around central vein and a decrease dead hepatocytes and kupffer cells. The revealed results coincides with the result of Kim et al. (2008) in which pretreatment of animals with HA along with APAP showed a
consistent preservation of hepatocyte around central vein, portal vein and the portal triad area. This might be due to HA absorbed through blood flows around central vein, portal vein and portal triad area and an easy chance to get conjugated to the cell wall, which could be the possible reason for protection of hepatocytes around central vein, portal vein and portal triad area (Kim et al., 2008). Previously, sulphated polysaccharide of Sargassum wightii exhibits a potent mitigating effect on oxidative liver injury induced by Cyclosporine A (Josephine et al., 2008). At the outset, the antioxidant potential isolated HA might play a key role in protection against APAP induced liver injury in rats.