Astaxanthin restricts weight gain, promotes insulin sensitivity and curtails fatty liver disease in mice fed an obesity-promoting diet

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1. Introduction

Obesity, an exaggeration of normal adiposity, is a global epidemic characterized by excess fat in adipose tissue and a spectrum of metabolic derangements like dyslipidemia, glucose intolerance and insulin resistance (IR) [1]. Ectopic fat storage occurs in obesity, particularly in the liver leading to a condition termed nonalcoholic fatty liver disease (NAFLD) characterized by varying degrees of liver injury that progresses from steatosis to steatohepatitis, fibrosis and fatty liver disease [9]. Consequently, in this study, we adopted the high fat-high fructose diet (HFFD). Fifteen days later, mice in each group were divided into two and treated with either ASX (6 mg/kg b.w.) in olive oil or olive oil alone. The mice were killed at the end of 60 days. Insulin sensitivity, markers of liver injury, inflammation and nitro-oxidative stress, antioxidants and cytochrome P4502E1 (CYP2E1) activity were assayed. Liver structural integrity was also assessed by histology with hemotoxylin and eosin and Masson’s trichrome stains. HFFD-fed mice registered significant increase in body weight and liver weight and displayed hyperglycemia, hyperinsulinemia and insulin resistance and elevated plasma aminotransferases. Lipid deposition, oxidative damage, defective antioxidant system and upregulated transforming growth factor-β (TGF-β1) expression were observed in HFFD-fed mice. ASX supplementation promoted insulin sensitivity and prevented liver injury by decreasing CYP2E1, myeloperoxidase, and nitro-oxidative stress and by improving the antioxidant status in them. Lipid deposition and increased TGF-β1 expression induced by HFFD were also abolished by ASX. This study provides new data showing the beneficial effects of ASX in obese mice.

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The study evaluated the effects of astaxanthin (ASX) in mice rendered obese by feeding an unbalanced diet. Adult male mice of body weight 25–35 g were fed either normal chow or a high fat-high fructose diet (HFFD). Fifteen days later, mice in each group were divided into two and treated with either ASX (6 mg/kg b.w.) in olive oil or olive oil alone. The mice were killed at the end of 60 days. Insulin sensitivity, markers of liver injury, inflammation and nitro-oxidative stress, antioxidants and cytochrome P4502E1 (CYP2E1) activity were assayed. Liver structural integrity was also assessed by histology with hemotoxylin and eosin and Masson’s trichrome stains. HFFD-fed mice registered significant increase in body weight and liver weight and displayed hyperglycemia, hyperinsulinemia and insulin resistance and elevated plasma aminotransferases. Lipid deposition, oxidative damage, defective antioxidant system and upregulated transforming growth factor-β (TGF-β1) expression were observed in HFFD-fed mice. ASX supplementation promoted insulin sensitivity and prevented liver injury by decreasing CYP2E1, myeloperoxidase, and nitro-oxidative stress and by improving the antioxidant status in them. Lipid deposition and increased TGF-β1 expression induced by HFFD were also abolished by ASX. This study provides new data showing the beneficial effects of ASX in obese mice.

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1. Introduction

Obesity, an exaggeration of normal adiposity, is a global epidemic characterized by excess fat in adipose tissue and a spectrum of metabolic derangements like dyslipidemia, glucose intolerance and insulin resistance (IR) [1]. Ectopic fat storage occurs in obesity, particularly in the liver leading to a condition termed nonalcoholic fatty liver disease (NAFLD) characterized by varying degrees of liver injury that progresses from steatosis to steatohepatitis, fibrosis and necrosis. Due to its prominent association with IR/obesity, NAFLD is regarded as the hepatic manifestation of metabolic syndrome [2].

Fat accumulation triggers free radical production and sensitizes the liver to additional necro-inflammatory insults, thus promoting disease progression [3]. Evidence for the close association between obesity and IR with low-grade inflammatory responses in liver has been consistently documented [4]. Production of proinflammatory cytokines and oxidative stress in the liver activate the resident immune cells of the liver like Kupffer cells and stellate cells, which mediate liver injury [5]. One such cytokine secreted by the hepatic stellate cells is transforming growth factor-β1 (TGF-β1) that has been implicated in fibrogenesis. TGF-β1, plays a central role in early stages of fibrogenesis by promoting the accumulation of a number of extracellular matrix proteins, specifically collagen by inhibiting its degradation and activating its biosynthesis [6].

Experimental studies have reported that animals fed a high fat plus high fructose diet (HFFD) for more than 2 months develop weight gain, dyslipidemia, moderate hyperglycemia, oxidative stress and IR [7,8]. Besides, consumption of a calorie-rich diet results in lipid accumulation, excess production of inflammatory cytokines, and macrophage infiltration that favour the progression of liver disease [9]. Consequently, in this study, we adopted the HFFD-fed mice as a model of fatty liver disease.

Natural compounds have been proposed as potential therapeutic agents in the prevention and/or treatment of number of diseases. Being aware of the increasing prevalence of obesity/IR, it is imperative to test and identify the possible therapeutic role of bioactive compounds that could be added to our diet. Astaxanthin (3,3′-dihydroxydiketo β carotene, ASX) is one such compound of interest used in this study. ASX is a red carotenoid pigment produced by marine-algal species like Haemotococcus pluvialis [10], Chlorella zofingiensis [11] and Scenedesmus obliquus [12]. It is ubiquitous in the marine environment and is responsible for the pinkish-red hue of the flesh of sea animals that ingest the marine algae. ASX is achieving commercial success in cosmetic industry and aquaculture and...
is widely used as a nutraceutical by athletes and sportsmen [13]. ASX is also known for its pharmacological activities that include antitumor, anti-ulcerative, anti-inflammatory and antigenotoxic activities [14].There has been considerable interest on the disease-preventing effects of ASX. This is revealed by mounting evidence, which show that addition of ASX reduces oxidative stress and inflammation in cardiovascular diseases [15], restores blood pressure in spontaneously hypertensive (SHR) rats [16], increases the endurance capacity in mice against exercise-induced fatigue [17] and improves memory in BALB/c mice [18]. Previous studies have shown its anti-obesity effects in mice fed high fat diet [19] and antidiabetic effects in db/db mice [20,21]. Although ASX has undergone such vast research by many scientists, its effects on fatty liver disease in obese mice have not yet been investigated. This study was therefore designed to investigate the effects of ASX on insulin sensitivity, hepatic function, inflammatory markers, TGF-β1 expression, lipid profile and oxidative and nitrosative stress in HFFD-fed mice. 

2. Materials and methods

2.1. Chemicals and kits

Astaxanthin (ASX) was purchased from Sigma-Aldrich Pvt. Ltd., St. Louis, MO, USA. The solvents and chemicals of analytical grade were purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India.

2.2. Animals

Adult male mus musculus albino mice of Swiss strain weighing 25–35 g were used for the study. The animals were procured from and maintained in the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai Nagar. They were individually housed under hygienic conditions (22–24 °C) in polypropylene cages under 12 h light/12 h dark cycle. The animals used in the present study were cared according to the principles and guidelines of the Institutional Animal Ethical Committee. After acclimatization for a period of 1 week, the animals were divided at random into four groups consisting of six mice each. The following groups were maintained for a total period of 60 days.

Group 1: CON—Animals received control diet and were orally administered olive oil (0.3 ml/kg b.w./day) from day 16 till the end of the experimental period.

Group 2: HFFD—Animals received HFFD and were orally administered olive oil (0.3 ml/kg b.w./day) from day 16 till the end of the experimental period.

Group 3: HFFD + ASX—Animals received HFFD and were orally administered ASX at a dose (6 mg/kg b.w./day) in olive oil from day 16 till the end of the experimental period.

Group 4: CON + ASX—Animals received control diet and were orally administered ASX in olive oil at a dose (6 mg/kg b.w./day) from day 16 till the end of the experimental period.

Food and water were provided ad libitum to animals. The dosage of ASX used in this study is based on a previous report which tested three doses of ASX (1.2, 6, and 30 mg/kg/day) for anti-obesity effects, among which ASX was effective at 6 and 30 mg/kg [19]. Hence the minimum dosage (6 mg/kg) was selected for our study. The body weight was measured every 4th day and food intake of the animals were recorded every 24 h. Food was replaced daily.

The high fat-high fructose diet (HFFD) of the following composition (g/100g) was prepared in the laboratory: 45.0 fructose, 10.0 ground nut oil, 10.0 beef tallow, 22.5 casein, 0.3

2.3. Glucose, insulin and insulin sensitivity indices

Plasma glucose and insulin were measured using kits obtained from Agappe Diagnostic Pvt. Ltd., Kerala and Accubind microwells, Monobind Inc., CA, USA, respectively. IR was assessed by computing homeostatic model assessment (HOMA) [22] and quantitative insulin sensitivity check index (QUICKI) [23]. The formulae used are given below:

\[
\text{HOMA} = \frac{\text{Insulin (µU/ml) × glucose (mM)}}{22.5}
\]

\[
\text{QUICKI} = \frac{1}{\log \text{ (glucose (mg/dl))+ log\text{ (insulin (µU/ml))}}}
\]

2.4. Aminotransferases, CYP2E1 and myeloperoxidase (MPO) activities

Alanine aminotransferase (ALT) and aspartate transaminase (AST) in plasma were measured using kits procured from Agappe Diagnostic Pvt. Ltd. Kerala. One International Unit is defined as µmol of pyruvate formed per minute under experimental conditions. CYP2E1 activity was determined by the method of Wu et al. by monitoring the oxidation of p-nitrophenol to p-nitrocatechol in the presence of NADPH and oxygen at 546 nm in a spectrophotometer [24]. Hepatic MPO activity was measured according to the method of Bradley et al. [25] with some modifications. The liver tissue was homogenized in 50 mM phosphate buffer containing 0.5% hexadecyltrimethyl- ammonium bromide, pH 6.0 (Sigma-Aldrich, St Louis, MO) and centrifuged at 2000 × g for 10 min at 4 °C. The supernatant (100 µl) was added to 1.9 ml of 10 mM phosphate buffer pH 60 and 1 ml of 1.5 M o-dianisidine hydrochloride (Sigma–Aldrich) containing 0.0005% (w/v) hydrogen peroxide. The changes in absorbance were recorded at 450 nm on a spectrophotometer for 5 min at 1 min intervals. Optical density units were converted into units of concentration using the molar absorptivity coefficient for oxidized to o-dianisidine hydrochloride (10,062 M⁻¹ cm⁻¹).

2.5. Nitro-oxidative stress markers

The oxidative stress markers such as thiobarbituric acid reactive substances (TBARS) [26], lipid hydroperoxides (LHP) [27] and protein carbonyl [28] were quantified. Total nitrite as an index of nitric oxide [29] and nitrosothiol content [30] were assayed. The activities of enzymatic (superoxide dismutate (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione-S-transferase (GST)) and non-enzymatic (vitamins C and E and reduced glutathione (GSH)) in the homogenate and liver were measured by methods outlined elsewhere [31].

2.6. Plasma and hepatic lipid profile

Lipids were extracted from the liver tissue according to the method of Folch et al. [32]. The content of triglycerides (TG), cholesterol and free fatty acids (FFA) were measured in plasma and liver by standard methods reported elsewhere [31].

2.7. Western blotting analysis of TGF-β1 in liver

Proteins were extracted from the frozen liver. Tissue homogenate in an ice-cold extraction buffer containing 20 mM Tris–HCl at PH 7.4, 150 mM NaCl (sodium chloride), 1 mM EDTA (ethylene diamine dichloroacetic acid), 0.5% Triton X-100, 0.1% SDS (sodium dodecyl sulphate), 1 mM PMSF (phenyl methysulphonyl fluoride and 10 µl of protease inhibitor cocktail) and centrifuged (10,000 × g, 15 min at 4 °C). After measuring the protein content by the method of Lowry et al. [33], samples (50 µg protein) were separated by 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred on to a nitrocellulose membrane at 80 mA for 1 h and 15 min. Membrane was then blocked overnight in a blocking buffer [3% bovine serum albumin in Tris-buffered saline pH 7.5 containing 0.1% Tween-20 (TBST)]. Then, the membrane was incubated with TGF-β1 antibody (rabbit polyclonal anti-TGF-β1, Santa Cruz Biotechnology (1:1000 dilution)) and agitated gently at 4 °C overnight. The membrane was washed with TBST for 5 min, and then incubated with anti-rabbit IgG conjugated with horseradish peroxidase (1:1500 dilution) for 2 h at room temperature. The protein bands were detected using diaminobenzidine (DAB) substrate. The bands were then scanned and the intensity was measured using densitometry software (AlphaEaseFC Software, Alphatech innotech Co., Johannesburg, South Africa). β-Actin was used as the internal control (1:1000 dilution).

2.8. Histology and immunohistochemistry

Four animals from each group were anesthetized using light ether, perfused with 10% formalin and the liver tissue was separated and placed in buffered formalin. They were later sectioned using a microtome (4–5 µm thickness), dehydrated in graded alcohol and embedded in paraffin. Sections were stained with hematoxylin and eosin (H and E) for Masson’s trichrome stain (MT). For immunohistochemistry, 4 µm paraffin sections were deparaffinized with xylene and rehydrated with graded concentrations of isopropyl alcohol. Separated sections were incubated with peroxidase blocking reagent for 10 min, rinsed with phosphate buffer and again incubated with power block solution for 10 min. Non-specific binding was minimized by leaving the
sections in 3% BSA in phosphate buffered saline for 30 min. Sections were incubated overnight with anti-TGF-β1 (1:250 dilution) antibody or with anti-DNP antibody (1:500 dilution). The sections were rinsed well with phosphate buffer and incubated with superenhancer reagent for 30 min. After rinsing with phosphate buffer, incubation was done with supersensitive polymer-horseradish peroxidase immunohistochemistry detection system (Biogenex laboratories, San Ramon, CA, USA). Sections were washed with buffer and incubated with DAB solution for 5 min. Sections were counterstained with hematoxylin and observed under the light microscope.

2.9. Statistical analysis

Values are expressed as means ± SD. Data within the groups were analyzed by one-way analysis of variance followed by Duncan’s multiple range test (DMRT). A value of P < 0.05 was considered statistically significant.

3. Results

3.1. Weight gain, liver weight and liver index.

Fig. 1 shows the body weights of animals during the experimental period. The final body weights (g) were CON, 31.3 ± 0.7; HFFD, 36.7 ± 0.7; HFFD + ASX, 33.0 ± 0.8; CON + ASX, 30.9 ± 1.0. The mean final body weight of HFFD-fed mice was significantly higher (17%) than control, while the value was significantly lower (11%) in HFFD + ASX mice than the mice fed HFFD alone. No significant difference in final body weight was observed between control and CON + ASX groups. The food intake (g/day/mice) were CON, 4.4 ± 0.31; HFFD, 4.3 ± 0.35; HFFD + ASX, 4.3 ± 0.29 and CON + ASX, 4.5 ± 0.33. There was no significant difference in food intake between different groups of animals. Table 1 shows the liver weight and liver index (Ll; liver weight/body weight x 100) of experimental animals at the end of the study period. Both liver weight and liver index were significantly higher in HFFD-fed mice when compared to control. However, increased liver weight and liver index observed in HFFD-fed mice were significantly brought down to near control levels when ASX was administered. The increase in liver index suggests hepatomegaly in HFFD-fed mice.

3.2. Glucose, insulin and insulin sensitivity indices

Plasma glucose and insulin levels were significantly higher in HFFD-fed mice than in the control group (P < 0.01) on the 16th and 60th days (Table 2). The addition of ASX from the 16th day till the end of the study was able to return glucose and insulin levels to those obtained in control mice. We performed the insulin sensitivity index HOMA and QUICKI, which also showed significant alterations in HFFD-fed mice indicating the development of IR on the 16th day itself. However, no statistical differences were observed in HOMA and QUICKI values among CON, CON + ASX and HFFD + ASX.

3.3. Aminotransferases, MPO and CYP2E1 activities

Table 3 shows the plasma levels of AST and ALT and hepatic CYP2E1 and MPO activities observed in experimental animals. The activities of all these enzymes were significantly higher in HFFD-alone fed group (P < 0.05) whereas the activities were near-normal in ASX-supplemented groups even when HFFD feeding was continued.

### Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CON</th>
<th>HFFD</th>
<th>HFFD + ASX</th>
<th>CON + ASX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver weight (g)</td>
<td>4.02 ± 0.21</td>
<td>4.80 ± 0.36^a</td>
<td>4.21 ± 0.27^b</td>
<td>4.11 ± 0.25</td>
</tr>
<tr>
<td>Liver index (g)</td>
<td>1.26 ± 0.06</td>
<td>1.76 ± 0.13^a</td>
<td>1.39 ± 0.08^b</td>
<td>1.27 ± 0.07</td>
</tr>
</tbody>
</table>

Values are means ± SD of 6 mice from each group. CON, control mice; HFFD, high fructose-high fat diet fed mice; HFFD + ASX, high fructose-high fat diet + astaxanthin (6 mg/kg b.w./day); CON + ASX, control + astaxanthin (6 mg/kg b.w./day).

### Table 2

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>15th day</th>
<th>60th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>80.11 ± 4.12</td>
<td>83.91 ± 4.52</td>
</tr>
<tr>
<td>HFFD</td>
<td>115.81 ± 7.61^a</td>
<td>134.81 ± 8.98^a</td>
</tr>
<tr>
<td>HFFD + ASX</td>
<td>–</td>
<td>98.77 ± 4.74^a</td>
</tr>
<tr>
<td>CON + ASX</td>
<td>–</td>
<td>82.66 ± 4.74</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>16.54 ± 1.23</td>
<td>17.42 ± 1.46</td>
</tr>
<tr>
<td>HFFD</td>
<td>25.33 ± 1.94^a</td>
<td>30.54 ± 2.63^a</td>
</tr>
<tr>
<td>HFFD + ASX</td>
<td>–</td>
<td>22.24 ± 1.76^a</td>
</tr>
<tr>
<td>CON + ASX</td>
<td>–</td>
<td>18.67 ± 0.96</td>
</tr>
<tr>
<td>HOMA</td>
<td>3.23 ± 0.18</td>
<td>3.60 ± 0.25</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.31 ± 0.002</td>
<td>0.32 ± 0.002</td>
</tr>
</tbody>
</table>

Values are means ± SD of 6 mice from each group. CON, control mice; HFFD, high fructose-high fat diet fed mice; HFFD + ASX, high fructose-high fat diet fed mice treated with astaxanthin (6 mg/kg b.w./day); CON + ASX, control mice treated with astaxanthin (6 mg/kg b.w./day).

### Table 3

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>CON</th>
<th>HFFD</th>
<th>HFFD + ASX</th>
<th>CON + ASX</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/ml)</td>
<td>0.27 ± 0.002</td>
<td>0.30 ± 0.002^b</td>
<td>0.31 ± 0.003</td>
<td></td>
</tr>
<tr>
<td>ALT (U/ml)</td>
<td>1.76 ± 18.67</td>
<td>4.74 ± 82.66</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD of 6 mice from each group. CON, control mice; HFFD, high fructose-high fat diet fed mice; HFFD + ASX, high fructose-high fat diet fed mice treated with astaxanthin (6 mg/kg b.w./day); CON + ASX, control mice treated with astaxanthin (6 mg/kg b.w./day).

^a Significant as compared to CON (P < 0.05; ANOVA followed by DMRT).

^b Significant as compared to HFFD (P < 0.05; ANOVA followed by DMRT).
Activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), CYP2E1 and MPO in experimental animals.

Table 3

<table>
<thead>
<tr>
<th>Parameters</th>
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<th>HFFD</th>
<th>HFFD + ASX</th>
<th>CON + ASX</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (IU/l)</td>
<td>47.61 ± 2.42</td>
<td>93.28 ± 6.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.62 ± 4.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.62 ± 2.61</td>
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<tr>
<td>ALT (IU/l)</td>
<td>51.20 ± 2.81</td>
<td>99.32 ± 7.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.96 ± 3.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.11 ± 3.43</td>
</tr>
<tr>
<td>CYP2E1 (Pmol min⁻¹ mg protein⁻¹)</td>
<td>3.77 ± 0.20</td>
<td>8.29 ± 0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.84 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.58 ± 0.18</td>
</tr>
<tr>
<td>MPO (μmol min⁻¹ mg protein⁻¹)</td>
<td>5.43 ± 0.29</td>
<td>8.93 ± 0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.09 ± 0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.41 ± 0.12</td>
</tr>
</tbody>
</table>

Values are means ± SD of 6 mice from each group. CON, control mice; HFFD, high fat-high fructose diet fed mice; HFFD + ASX, high fat-high fructose diet fed mice treated with astaxanthin (6 mg/kg b.w./day); CON + ASX, control mice treated with astaxanthin (6 mg/kg b.w./day).

<sup>a</sup> Significant as compared to CON (P<0.05; ANOVA followed by DMRT).

<sup>b</sup> Significant as compared to HFFD (P<0.05; ANOVA followed by DMRT).

Table 4

<table>
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<tr>
<th>Parameters</th>
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<th>HFFD</th>
<th>HFFD + ASX</th>
<th>CON + ASX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Plasma</td>
<td>2.50 ± 0.15</td>
<td>3.57 ± 0.21</td>
<td>2.80 ± 0.19</td>
<td>2.58 ± 0.14</td>
</tr>
<tr>
<td>Liver</td>
<td>3.20 ± 0.17</td>
<td>5.83 ± 0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.98 ± 0.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.07 ± 0.16</td>
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<tr>
<td>Triglycerides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>0.46 ± 0.03</td>
<td>0.71 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.50 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.53 ± 0.03</td>
</tr>
<tr>
<td>Liver</td>
<td>5.48 ± 0.32</td>
<td>8.02 ± 0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.40 ± 0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.20 ± 0.33</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>2.45 ± 0.15</td>
<td>3.69 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.89 ± 0.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.53 ± 0.16</td>
</tr>
<tr>
<td>Liver</td>
<td>6.47 ± 0.32</td>
<td>9.81 ± 0.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.57 ± 0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.33 ± 0.34</td>
</tr>
</tbody>
</table>

Values are means ± SD of 6 mice from each group. CON, control mice; HFFD, high fat-high fructose diet fed mice; HFFD + ASX, high fat-high fructose diet fed mice treated with astaxanthin (6 mg/kg b.w./day); CON + ASX, control mice treated with astaxanthin (6 mg/kg b.w./day).

<sup>a</sup> Significant as compared to CON (P<0.05; ANOVA followed by DMRT).

<sup>b</sup> Significant as compared to HFFD (P<0.05; ANOVA followed by DMRT).

3.4. Plasma and liver lipids

Dyslipidemia was noted in HFFD-fed mice. This included 54% increase in TG, 42% increase in cholesterol and 50% increase in FFA compared to control. Similarly lipid analysis in the liver also revealed excess storage. The total lipid levels (mg/g tissue) were CON, 42.64 ± 2.4; HFFD, 51.70 ± 4.3; HFFD + ASX, 44.83 ± 2.8; CON + ASX, 41.76 ± 2.6. The total lipid content in the liver of HFFD-fed mice was increased 1.2-fold when compared with mice fed control diet whereas in ASX + HFFD, the value did not differ significantly from control. Levels of TG, total cholesterol and FFA were significantly higher by 46%, 82% and 50%, respectively in HFFD-fed mice. ASX treatment of HFFD-fed mice restored plasma and liver lipid levels to near-normal values (Table 4). There were no significant differences in lipid levels between CON and CON + ASX groups.

Table 5

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CON</th>
<th>HFFD</th>
<th>HFFD + ASX</th>
<th>CON + ASX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBARS (μmol/dl)</td>
<td>0.92 ± 0.05</td>
<td>2.19 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.33 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.96 ± 0.06</td>
</tr>
<tr>
<td>LHP (μmol/dl)</td>
<td>0.99 ± 0.04</td>
<td>2.16 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.22 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.92 ± 0.05</td>
</tr>
<tr>
<td>PC (μmol/dl)</td>
<td>1.78 ± 0.14</td>
<td>2.25 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.84 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.69 ± 0.14</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TBARS (nmol/mg protein)</td>
<td>1.26 ± 0.07</td>
<td>2.22 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.61 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.28 ± 0.08</td>
</tr>
<tr>
<td>LHP (nmol/mg protein)</td>
<td>1.36 ± 0.07</td>
<td>2.72 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.75 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.39 ± 0.08</td>
</tr>
<tr>
<td>PC (nmol/mg protein)</td>
<td>1.15 ± 0.06</td>
<td>2.66 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.75 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.18 ± 0.07</td>
</tr>
</tbody>
</table>

Values are means ± SD of 6 mice from each group. CON, control mice; HFFD, high fat-high fructose diet fed mice; HFFD + ASX, high fat-high fructose diet fed mice treated with astaxanthin (6 mg/kg b.w./day); CON + ASX, control mice treated with astaxanthin (6 mg/kg b.w./day).

<sup>a</sup> Significant as compared to CON (P<0.05; ANOVA followed by DMRT).

<sup>b</sup> Significant as compared to HFFD (P<0.05; ANOVA followed by DMRT).

3.5. Oxidative stress markers

The levels of oxidative stress markers such as LHP, TBARS and PC were measured to study the extent of oxidative damage and are presented in Table 5. The levels were significantly increased in HFFD group. ASX treatment of HFFD-fed mice controlled the levels of lipid peroxidation products and protein oxidation products. The levels of nitrosative stress markers such as total nitrite and nitrosothiol are tabulated in Table 6. Significantly higher levels of nitrosothiol (P<0.05) and lower levels of nitrite (P<0.05) were observed in HFFD-fed mice. ASX administration brought back the levels of nitrosothiol and nitrite to near-normal.

The activities of enzymatic and non-enzymatic antioxidants in the hemolysate and liver of animals in each group are shown in Tables 7 and 8, respectively. The activities of SOD, CAT, GPX and GST in HFFD-fed group were significantly decreased in circulation (by 62%, 36%, 43% and 33%, respectively) and in the liver (by 26%, 50%, 23% and 36%, respectively) as compared to control mice. The concentration of non-enzymatic antioxidants followed a similar trend both in plasma and liver. Co-treatment with ASX prevented the decline in antioxidant system in circulation and liver.
3.6. Blotting analysis of TGF-β1

Fig. 2(a) represents the protein expression of TGF-β1 in liver while Fig. 2(b) represents the results of the densitometry analysis. Increased expression of TGF-β1 protein, observed in HFFD-fed mice, was reduced significantly when ASX was supplemented. On the other hand, the protein expression was similar in ASX-untreated control mice and ASX-treated control mice.

3.7. Histological changes

Figs. 3 and 4 show the histological analysis of liver by H and E and MT stain, respectively. H and E staining revealed necrosis and mild inflammatory cell infiltration (active hepatitis) in HFFD-fed mice (Fig. 3B). Furthermore, liver of HFFD-mice shows microvesicular and macrovesicular fatty vacuoles. On the other hand, liver from mice administered ASX during HFFD feeding shows reduction in inflammatory cell infiltration, fat accumulation and necrosis. MT staining of liver section from HFFD-fed mice revealed areas of necrosis involving all the zones and also deposition of collagen (Fig. 4B) compared to control (Fig. 4A). These abnormalities associated with HFFD feeding were attenuated by ASX treatment. ASX-administered control mice showed normal collagen distribution in liver as that of control (Fig. 4A and D).

3.8. Immunohistochemistry

Figs. 5 and 6 are the photomicrographs showing the localization of DNP–protein adduct and TGF-β1 protein, respectively in liver. The intensity of DNP and TGF-β1 staining was more pronounced in HFFD-fed mice than in control. It is noteworthy that there was a marked reduction in DNP–protein adduct and TGF-β1 immunoreactivity in the liver of HFFD-fed mice treated with ASX. There was no apparent difference in the immunoreactivity between CON and CON + ASX.

4. Discussion

The major findings of the study are the following: (1) ASX markedly decreased the weight gain and limited the hepatomegaly

Table 7

Activities of enzymatic antioxidants in hemolysate and liver of experimental animals.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CON</th>
<th>HFFD</th>
<th>HFFD + ASX</th>
<th>CON + ASX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemolysate</td>
<td></td>
<td></td>
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<tr>
<td>SOD (U/mg Hb)</td>
<td>3.98 ± 0.20</td>
<td>1.51 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.50 ± 0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.81 ± 0.30</td>
</tr>
<tr>
<td>CAT (μmol of H₂O₂ consumed min⁻¹ mgHb⁻¹)</td>
<td>39.90 ± 2.02</td>
<td>25.31 ± 1.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.51 ± 2.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.42 ± 2.26</td>
</tr>
<tr>
<td>GPX (μmol of GSH consumed min⁻¹ mgHb⁻¹)</td>
<td>8.10 ± 0.43</td>
<td>4.60 ± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.50 ± 0.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.06 ± 0.35</td>
</tr>
<tr>
<td>GST (μmol of CDNB conjugate consumed min⁻¹ mgHb⁻¹)</td>
<td>5.35 ± 0.30</td>
<td>2.71 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.78 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.35 ± 0.29</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>4.94 ± 0.27</td>
<td>3.64 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.37 ± 0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.79 ± 0.26</td>
</tr>
<tr>
<td>CAT (μmol of H₂O₂ consumed min⁻¹ mg protein⁻¹)</td>
<td>48.72 ± 2.75</td>
<td>24.44 ± 2.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.44 ± 2.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.43 ± 2.45</td>
</tr>
<tr>
<td>GPX (μmol of GSH consumed min⁻¹ mg protein⁻¹)</td>
<td>6.01 ± 0.34</td>
<td>4.58 ± 0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.39 ± 0.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.90 ± 0.34</td>
</tr>
<tr>
<td>GST (μmol of CDNB conjugate formed min⁻¹ mg protein⁻¹)</td>
<td>5.39 ± 0.31</td>
<td>3.44 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.80 ± 0.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.29 ± 0.34</td>
</tr>
</tbody>
</table>

Values are means ± SD of 6 mice from each group. CON, control mice; HFFD, high fat-high fructose diet fed mice; HFFD + ASX, high fat-high fructose diet fed mice treated with astaxanthin (6 mg/kg b.w./day); CON + ASX, control mice treated with astaxanthin (6 mg/kg b.w./day).

<sup>a</sup> Significant as compared to CON (P<0.05; ANOVA followed by DMRT).

<sup>b</sup> Significant as compared to HFFD (P<0.05; ANOVA followed by DMRT).

Table 8

Levels of non-enzymatic antioxidants in plasma and liver of experimental animals.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CON</th>
<th>HFFD</th>
<th>HFFD + ASX</th>
<th>CON + ASX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td></td>
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<tr>
<td>GSH (mg/dl)</td>
<td>18.64 ± 0.95</td>
<td>10.48 ± 0.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.55 ± 1.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.38 ± 1.07</td>
</tr>
<tr>
<td>Vitamin C (mg/dl)</td>
<td>1.54 ± 0.09</td>
<td>0.96 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.33 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.45 ± 0.07</td>
</tr>
<tr>
<td>Vitamin E (mg/dl)</td>
<td>1.33 ± 0.07</td>
<td>0.87 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.18 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.27 ± 0.07</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSH (mg/100 g tissue)</td>
<td>86.58 ± 4.77</td>
<td>64.98 ± 5.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.81 ± 4.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.28 ± 4.82</td>
</tr>
<tr>
<td>Vitamin C (μg/mg protein)</td>
<td>2.23 ± 0.12</td>
<td>1.15 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.98 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.19 ± 0.13</td>
</tr>
<tr>
<td>Vitamin E (μg/mg protein)</td>
<td>4.13 ± 0.23</td>
<td>2.56 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.62 ± 0.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.98 ± 0.25</td>
</tr>
</tbody>
</table>

Values are means ± SD of 6 mice from each group. CON, control mice; HFFD, high fat-high fructose diet fed mice; HFFD + ASX, high fat-high fructose diet fed mice treated with astaxanthin (6 mg/kg b.w./day); CON + ASX, control mice treated with astaxanthin (6 mg/kg b.w./day).

<sup>a</sup> Significant as compared to CON (P<0.05; ANOVA followed by DMRT).

<sup>b</sup> Significant as compared to HFFD (P<0.05; ANOVA followed by DMRT).

Fig. 2. Expression of transforming growth factor β1 (TGF-β1) in liver of experimental animals. (a) Expression of TGF-β1 in liver of experimental animals by immunoblotting. Lane 1, CON; Lane 2, HFFD; Lane 3, HFFD + ASX (6 mg/kg b.w./day); Lane 4, CON + ASX (6 mg/kg b.w./day). (b) Results of the densitometry analysis. For quantitative assessment, the band intensity was measured in a densitometer and normalised with β-actin values. The changes relative to control are expressed in the bar diagram. Values are means of 5 determinations. *Significant compared to control; †significant compared to HFFD.
induced by the high calorie diet; whole body insulin sensitivity was substantially improved and (2) ASX ameliorated oxidative insult, liver injury and inflammation imposed by the high calorie diet.

The rise in bodyweight upon the consumption of HFFD has been previously reported [8] and is expected from the increased energy intake from the high calorie diet. The rise in liver weight might be due to excess lipid/collagen accumulation. Confirming earlier studies [7,8], HFFD-fed mice developed significant elevation in both glucose and insulin levels indicating the development of IR. The presence of IR is further indicated by higher values of HOMA-IR and lower values of QUICKI. Animals fed HFFD showed hepatic injury resulting in the release of cellular enzymes such as AST and ALT into circulation.

Accumulation of lipids, increases in cholesterol, TG and FFA in liver of HFFD-fed mice were observed. Histological evaluation of the liver also showed fatty changes of micro- and macrovesicular types consistent with biochemical measurements. Diet rich in fructose induces the lipogenic pathways by activating the gene expression of sterol retinal binding protein-1, fatty acid synthase and acetyl CoA carboxylase and by decreasing the activities of enzymes of fatty

![Photomicrograph of liver sections stained with hematoxylin and eosin 40×.](image)

(A) Normal liver histology of control mice. (B) Liver section from HFFD-fed mice. The arrows show fat vacuoles of microvesicular and macrovesicular types. (C) Liver section from HFFD + ASX (6 mg/kg b.w./day) mice. The arrows represent reduction in lipid deposition; microvesicular type of fatty changes is minimal. (D) Normal liver histology of control + ASX mice (6 mg/kg b.w./day).

![Photomicrograph of liver sections stained with Masson trichrome 40×.](image)

(A) Normal liver histology of control mice. (B) Liver section of HFFD-fed mice. Arrows show the increased deposition of collagen around the focal areas. (C) Liver section of HFFD + ASX (6 mg/kg b.w./day). The arrows represent marked decrease in the deposition of collagen around central vein. (D) Normal liver mice treated with ASX (6 mg/kg b.w./day) shows normal deposition of collagen around central vein.
Excess storage of lipids induces a state of lipotoxicity that is related to the formation of fatty acyl COA, diacylglycerol and other potentially toxic lipid metabolites which activate deleterious stress-sensitive pathways that may suppress the insulin signal cascade [34]. In addition, cholesterol or saturated fatty acid accumulation can elicit stress in the endoplasmic reticulum and trigger the unfolded protein response, an event that down regulates insulin signaling [35].

The reversion of dyslipidemia and prevention of lipid accumulation in liver by ASX suggest that it might act to shift lipid acid oxidation resulting in VLDL-TG secretion causing a rise in liver TG [7]. Furthermore, excess delivery of FFA from the insulin resistant adipocytes to the liver could result in esterification of TG that contributes to the development of liver steatosis.

Fig. 5. Immunohistochemical localization of DNP–protein adducts in liver of experimental animals. DNP-immunostaining is increased in liver of HFFD-fed mice (B) and is reduced in ASX (6 mg/kg b.w./day) treated HFFD-fed mice (C). Both control (A) and ASX (6 mg/kg b.w./day) treated control mice (D) show negligible expression of DNP adducts.

Fig. 6. Representative photomicrograph for the accumulation of TGF-β1 protein in liver of animals from each group. TGF-β1 immunostaining is increased in liver of HFFD-fed mice (B) and this expression is markedly reduced in ASX (6 mg/kg b.w./day) treated HFFD-fed mice (C). Both control (A) and ASX (6 mg/kg b.w./day) treated control (D) mice show minimal expression for this protein.
metabolism from synthesis and storage to oxidation. Further, the suppression of lipotoxicity by ASX could mediate the improvement of insulin sensitivity by deactivating the stress-sensitive pathways.

Reducing energy intake and increasing energy expenditure are fundamental to the treatment of obesity. It is important to note that the food intake was similar in all the groups but HFFD-mice (with or without ASX) received more calories than control mice. ASX has been shown to promote fatty acid metabolism in skeletal muscle during exercise via carnitine palmitoyltransferase (CPT I) activation [36]. Ikeuchi et al. [17,19] also noted that ASX does not affect food intake but lowers body weight in obese mice possibly by increasing energy expenditure. This explains the mechanism of weight reduction and increased energy expenditure by ASX in HFFD-mice.

Oxidative lipid and protein modification in HFFD-fed mice could occur through the generation of free radicals from glucose by mechanisms like autooxidation, protein glycation and polyol pathway [37]. The formation of 5-nitrosothiol by the reaction between thiols especially GSH and peroxynitrite, a highly reactive nitrogen species causes depletion of GSH and promotes oxidative stress. Further, carbonylation and nitrosylation of proteins alter the structure and function of cellular and membrane proteins and impair cell function [38,39]. Piconi et al. [40] reported that protein modification/damage could reduce insulin activity.

Liver cytotoxicity and oxidative burden inflicted by HFFD is suggestive of inefficient antioxidant system. The antioxidant enzymes such as CAT and GPX are themselves susceptible to reactive oxygen species (ROS) attack [41]. The decreased SOD activity in HFFD-fed animals may be attributed to inactivation of the enzyme due to glycation of Lys122 and Lys128 residues [42]. Decreased GSH, vitamins E and C could be due to increased utilization to combat free radicals and/or decreased regeneration from their oxidized forms.

ASX is recognized to be a free radical scavenger as well as a chain breaking antioxidant [43]. The presence of hydroxyl and keto moieties on each ionone ring of ASX confer unique features like higher antioxidant activity and more polar configuration than other carotenoids [44]. Transmembrane orientation of ASX is likely responsible for its antioxidant properties and its biological effects. Goto et al. [45] reported that polar ring of ASX can scavenge the ROS near the membrane surface, while the polyene chain would inhibit the radical chain reaction into the membrane.

Glucose-induced ROS production results in the activation of transcription factor NF-kB (nuclear factor kappa B) and subsequent expression/production of cytokines which are documented to play an important role in downregulation of insulin signal cascade [46]. Further, insulin receptor gene expression requires efficient transcription of certain factors [47] which are modulated by GSH levels. Thus ASX, by its antioxidant activity and by replenishing GSH, promotes insulin sensitivity and alleviates oxidative stress and liver damage. ASX has also been shown to prevent mitochondrial ROS-induced modification of CPT I via its antioxidant effect [36].

CYP2E1, a member of cytochrome P450 enzymes, is a proxidant enzyme and is the major microsomal source of superoxide. CYP2E1 upregulation could be responsible for the development of liver injury by its ability to induce ROS production in HFFD-mice [48]. Since CYP2E1 is normally suppressed by insulin, it is conceivable that reduced insulin action would result in increased CYP2E1 activities. In this context it is important to look at the studies by Schattenberg et al. [49], which indicate the potential role of C-Jun-N terminal kinase-dependent downregulation of insulin signaling cascade by CYP2E1 overexpression in a dietary mouse model of steatohepatitis. ASX administration decreased CYP2E1 activity to near-normal, which is a strong evidence for the antioxidant role of ASX and gives a possible clue to its insulin sensitivity effects.

MPO originates from activated neutrophils and monocytes and is an indicator for inflammatory cell infiltration and tissue injury. Increased MPO activity suggests neutrophil migration and activation and is likely to account for the increased parenchymal injury and hepatitis in HFFD-fed mice. On the contrary, ASX produced inhibition of MPO activity that reflect the reduction in migration of neutrophils and other inflammatory cells in the liver, thereby alleviating liver damage.

TGF-β1 is a profibrogenic cytokine secreted by the activated stellate cells of the liver. Elevated glucose, ROS and lipid peroxidation products increase TGF-β1 causing cell death, inflammation and fibrosis [50]. Thus, the overexpression of TGF-β1 in HFFD-fed mice may arise from ROS generation and glucotoxicity which is prevented by ASX treatment. There is a report showing that ASX protects mesangial cells in culture from glucose-induced ROS production, the activation of transcription factors and cytokine expression [46]. Thus, we interpret that ASX acts by suppressing the expression of TGF-β1, a stimulator of fibrogenic process. Reduction in collagen in liver section of HFFD + ASX animals is affirmative with its antifibrogenic role. Although, no studies have identified the antifibrogenic effects of ASX, the anti-inflammatory action has been increasingly recognized. For instance, ASX but not vitamin E was able found to reduce swelling of rat paw in an inflammatory model [51].

One limitation of the study is that the bioavailability of ASX has not been tested, but this has been reported by several investigators [21,52]. Rufer et al. [52] studied ASX concentration and distribution of isomeric forms in an human intervention study and observed that ASX is absorbed into plasma without any appreciable metabolic transformation. Animal studies also observed high bioavailability of ASX. ASX is shown to be metabolized in the liver and accumulates in tissues (liver and kidney) of mice [21].

Human and animal studies have shown that western diet which is high in fat and sugar is associated with obesity and other related metabolic diseases [53]. The composition of HFFD mimics that of a typical unhealthy western diet [7]. We used HFFD to induce obesity in mice which serve as a model to investigate the possible protective role of ASX. ASX exerts anti-hyperglycemic effects in db/db mice model as well [20,21]. ASX has been shown to preserve β-cell function, reduce glucose toxicity, prevent renal cell damage and delay the progression of diabetic nephropathy in db/db mice [21].

The present observations reflect that the high calorie diet, rich in fat and fructose could be a driving force for the disease process in terms of oxidative stress, inflammation and fibrosis. With the addition of ASX, it is possible to prevent an early liver injury and activation of the fibrogenic process. ASX is of interest to many recent researchers because of the anti-inflammatory and antiatherogenic effects, which stem from its antioxidant role. Besides antioxidant function, other effects specifically receptor activation or interaction with endogenous bioactive factors by ASX must also be considered in future. ASX is one of the ingredients in a number of health products. In this vein, clinical studies have revealed that healthy adults can consume 6 mg per day from H. pluvialis algal extract [54].

In summary, we have used a dietary model of fatty liver disease associated with IR, which mimics the human NAFLD, to show that ASX restricts weight gain, promotes insulin sensitivity and curtails liver disease. These changes were verified by examining the reduction in CYP2E1 activity, oxidative stress, TGF-β1 expression and MPO activity. These abilities of ASX make it a potential drug for preventing liver disease arising from IR/obesity.

Conflict of interest

The authors declare that there are no conflicts of interest.
Acknowledgment

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References


