EVALUATION OF ANTIOXIDANT AND ANTIHYPERLIPIDEMIC ACTIVITY OF INDIAN GOOSEBERRY (Emblica officinalis) FRUIT IN HIGH FAT-FED RABBITS

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ABSTRACT

Hyperlipidaemia leads to various diseases including cardiovascular and diabetes mellitus, etc. Among the many reasons for the abnormal lipid profile, high fat intake and sedentary life style are the main reasons for hypercholesterolemia. In the present study, a total of 25 (n=25) rabbits were used and were randomly divided into five groups which received different treatments. Group I served as control, group II received a measured quantity of Emblica officinalis (EO) powder, group III received high fat diet (HFD), group IV received HFD and EO powder and group V received HFD and the standard drug atorvastatin for eight weeks. To investigate the effects of EO powder, blood and tissue (liver and kidney) samples were taken at the end of the eight weeks of experimentation and serum was evaluated for various blood plasma lipid parameters and antioxidant enzymes of liver and kidney. There was a significant increase in all the lipid parameters except for HDL in the group of rabbits receiving HFD; HDL level decreased significantly (P< 0.05). High fat intake may have resulted in the production of free radicals which created an oxidative stress and lipid peroxidation ultimately leading to abnormal lipid profile. Antioxidant enzymes, such as Superoxide dismutase (SOD), Catalase (CAT) and Glutathione (GSH) were significantly reduced in the HFD group indicating clearly that high fat diet leads to oxidative stress. Emblica officinalis relieved the oxidative stress of liver and kidney tissues in group IV indicating that EO powder has the potential to inhibit the oxidative stress and lipid peroxidation because of poly-phenols, flavonoids, ascorbic acid and other chemicals present in it. Further, it is concluded that although statins are useful in reducing blood plasma lipid profile, they simultaneously have many side effects. The findings of the present study reveal that EO powder is useful in reducing hyperlipidaemia and tissue damage.

Keywords: Emblica officinalis, Atorvastatin, Hyperlipidemia, Lipid Profile, Medicinal Plants.

INTRODUCTION

High blood cholesterol level is the major reason for various diseases including nervous and cardiovascular disorders (Goldstein and Brown, 2015). There are a number of reasons for this hyperlipidemic condition including high fat diet, sedentary lifestyle, genetics, kidney problems and liver disease. Several factors, such as increased age, lack of exercise, obesity, smoking, excessive alcohol intake, diabetes, low thyroid and Cushing's syndrome increase the risk to be hyperlipidemic (Kawasumi et al., 2014). There are several epidemiological findings that indicate high blood cholesterol, clotting factors and oxidation of lipoproteins lead to the formation and rupture of atherosclerotic plaque (Cappelli-Bigazzi et al., 1997), decreasing the oxygenated blood flow towards vital organs leading to angina, heart attack, stroke and other serious complications (Herron et al., 2004; Varbo et al., 2014).

Cholesterol lowering drugs of different classes (Statins, Niacin, Bile-acid binding resins, Omega-3 fatty acids, Fibrates, Cholesterol absorption inhibitor, etc.) are frequently used to avoid damages of hyperlipidaemia. Mostly these medicines reduce low density lipoproteins (LDL) level and some also increase the high density lipoproteins (HDL) level in blood circulation through different mechanisms (Illingworth, 1987). Although these drugs are very beneficial to lower the bad lipid profile parameters, they also have adverse side effects including memory loss, depression, insomnia, itching and headache, etc. (Kureishi et al., 2000; Pahan, 2006).

Man has been using the natural herbal drugs since centuries. Researchers are trying their best to investigate natural plants for their role for the cure of different ailments (Anjum et al., 2014). There are some evidences that some plants have immense potential for medicinal use. The use of several plants has been reported for the treatment of hyperlipidaemia. Natural products must preferably be used to lower the blood lipids. These products not only rescue the body from hyperlipidaemia but also improve the function of other body organs. For example, the fruit of Emblica officinalis (EO) is used to manage the blood glucose levels but simultaneously it improves the digestive system and
protects the body from harmful microbes (Ahmad et al., 1998; Khan, 2009).

**Emblica officinalis** (EO), a deciduous ornamental tall tree (approximately 60 ft. tall), is a member of family Euphorbiaceae, locally known as “amla”. It is a traditional medicinal plant native to tropical parts of Southeast Asia. Due to its medicinal and economic importance, it is cultivated commercially in India, China and some other countries (Sairam et al., 2002). All parts of this plant are reported to be used for preparation of various herbal medicines to cure various ailments. Decoction and infusion of leaves and seeds of this plant are commonly used (Kumar et al., 2012b). These extracts and distillates of leaves and seeds are reported to have bactericidal, anti-inflammatory and antipyretic properties and are used as natural mouth wash and also applied against cold, dysentery, fever and urine retention (Jain et al., 2014). The flowers and roots are used as refrigerant, aperient and emetic (Abbasi et al., 2010) and their extracts are used for the treatment of gonorrhea, diarrhoea and mouth inflammations (Kumar et al., 2012a). Seeds and seed oil of this plant are used to cure asthma, skin complications and bronchitis (Jain et al., 2015; Khan, 2009).

**Emblica officinalis** fruit is considered diuretic and laxative while fruit juice is used to cure jaundice, dyspepsia and coughs (Kumar et al., 2012b). The powdery paste of the fruit is an expectorant and astringent for the treatment of various diseases like indigestion, heart problems and nasal congestion, etc. (Jain et al., 2014). The juice from leaves and fruit act as antimicrobial agent to cure sores and wounds (Ahmad et al., 1998). Leaves and fruit of EO plant are reported to be rich in tannin, ascorbic acid, amalic acid, flavonoids and ellagic acid, etc. (Scartezzini et al., 2006). There are several reports that various medicinal plants possess antioxidative properties. Antioxidant enzymes protect body and tissues by various mechanisms. Superoxide dismutase (SOD) converts harmful “superoxide anions (O₂⁻)” to H₂O₂ and hydrogen peroxide is catalysed by catalase (CAT) to water and O₂. Further, glutathione detoxify H₂O₂ and other organic hydro-oxides, thus protect tissues and relieves the oxidative stress (Bazmandegan et al., 2017). Flavonoids are widely distributed in the plant phenolic compounds, known to have more than 4000 kinds of individual substances (Qasim et al., 2017). Plants use these flavonoids to prevent oxidative damage by suppressing the free radicals and reactive oxygen species (ROS) produced by sunlight. Dietary polyphenols enhance the stability of LDL against oxidation (Scartezzini et al., 2006). Several studies have reported that oxidized LDL, in turn, plays an important role in atherosclerosis and cardiovascular disease (CVD). Epidemiological studies have proven that intake of antioxidant rich foods are inversely proportional to the CVD (Shyamala et al., 2003). Most of the phenolic compounds have potential to act as antioxidants because they possess conjugated ring structure and the hydroxyl group. Free radicals, hydrogen peroxide leads to oxidative stress which is cytotoxic to the tissues and antioxidants from flavonoids protect tissues and make complexes and hydrogenate free radicals and convert them to neutral compounds (Binti Anzian et al., 2017).

Keeping in view the broad beneficial aspects of EO fruit, the present study was conducted to evaluate the antioxidative and hypolipidemic potential of this fruit in high fat fed rabbits. Furthermore, anti-hyperlipidemic and antioxidative efficiency of EO fruit powder was also compared with a standard cholesterol lowering drug.

### MATERIALS AND METHODS

**Animals:** The study was conducted at the Department of Wildlife & Ecology, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. Twenty five large rabbits (Oryctolagus cuniculus) were used for the study. Their mean ages was 50±10 days and mean body weight was 1.75±0.25 Kg either sex. Animals were acclimatized for 10 days in the University animal enclosures under standard conditions before the start of the experiment. The animals were fed on normal diet consisting of fodder and grams in sufficient quantity throughout the duration of the experiment and water was provided ad libitum.

**Emblica officinalis (EO):** EO fruit was purchased from a local market, identified and authenticated by a professional botanist, washed with water, air dried and ground along with the seeds.

**Atorvastatin:** Atorvastatin is a standardized medication for the reduction of circulatory blood cholesterol level. This drug was purchased from a local medical store. Atorvastatin belongs to the broad spectrum medicines used as a source of statin. A dose of 2.5mg/kg/day in rabbits was used to compare the efficiency of the test drug, EO, with the standard drug according to Ahmed et al., (2015).

**High Fat Diet:** High fat diet (HFD) was made as described by Anjum et al., (2014) to induce hypercholesterolemia by feeding a daily dose of 10 ml/kg/body weight orally as described by Ghuffar et al. (2014).

**Study Plan:** The experiment was performed for eight weeks. The animals were weighed, numbered and randomly separated into five groups with five animals per group as follows:

Group I served as a control group which received only Normal Saline. Group II was fed on normal diet and received a daily dose of 600mg/Kg of EO. Group III was fed on normal diet and a daily dose of HFD. Group IV received a daily dose of HFD and the test drug
Dose Administration: The powdered EO dose and Atorvastatin dose were measured and mixed with normal saline, while HFD was directly fed to the animals by an intra-gastric feeding tube as described by Dhuley (1999).

Serum Analysis: Feeding and maintenance of experimental animals was monitored very carefully until the end of the study. After eight weeks, 2ml blood was collected from marginal ear vein with the help of a capillary tube from each group to quantify the various parameters of lipid profile. Animals were fasted for at least 14 hours before the blood collection. The collected blood samples were centrifuged at 2500 x g at 20 °C for 10 minutes and serum thus obtained was stored at -20 °C as described by Anjum et al., (2014) and Yaqub et al., (2018). Serum was analyzed by calorimetric test for quantitative estimation of total cholesterol (TC), LDL, HDL, and Triglycerides (TG).

Estimation of Antioxidants: The rabbits were sacrificed and liver and kidneys were separated after dissection for the estimation of antioxidants. Tissues were homogenized in Phosphate-buffered saline (PBS) 7.4 pH and homogenate was sonicated and centrifuged at 20000 g for 15 min at 4 °C temperature. The supernatant was divided into two parts; one aliquot was stored at -20 °C while the other was used for estimation of antioxidants. Superoxide dismutase, Catalase and Glutathione peroxidase activity was measured as described by Sun et al., (1988), Kawamura et al., (1991), Paglia and Valentine (1967), respectively.

Statistical Analysis: Kolmogorov Smirnov’s test was applied to evaluate the normal distribution of data. Data were presented as mean ±S.D. The data were analyzed by one-way analysis of variance (ANOVA), followed by Dunnette’s comparison tests using statistical package SPSS (Version 13.0 SPSS Inc., Chicago, IL, USA). Results with P<0.05 were considered statistically significant.

RESULTS

A significant increase in all the lipid parameters, except for HDL was observed following the administration of HFD when compared to control (Table 1). Mean TC value amounted to 105.79±3.37 mg/dl in control rabbits, whereas in fat-fed rabbits this value amounted to 271.38±5.88mg/dl after eight weeks of the experimentation period. In the control group, mean values of LDL and TG were 63.32±2.34 mg/dl and 72.54±1.98 mg/dl, respectively; however, a significant increase was noted in both of these parameters in the HFD-fed animal group. Both LDL and TG levels decreased significantly (P< 0.05) due to administration of the test drug. The TG in the HFD-fed group was significantly higher (P< 0.05) as compared to the control group (72.54±1.98 mg/dl). Triglycerides level also decreased almost to the standard drug treatment level due to EO treatment. HDL decreased significantly (17.93±1.84mg/dl) with the increased HFD intake. HDL level was observed higher in rabbits when they were administrated EO with normal feed. EO maintained the HDL at significant (29.280±1.39 mg/dl) high level even when administrated with HFD as shown in Figure 1a. High fat diet led to significant decrease (P< 0.05) of SOD, CAT and GSH in liver and kidney of HFD group as shown in Table 2. *Emblica officinalis* powder significantly increased the content of antioxidant enzymes in liver and kidney tissue. Results also suggest that EO has better antioxidant potential than standard drug, statin; however, it was also noted that statin also relieved the oxidative stress of liver and kidney tissues in HFD group. The study revealed that concomitant administration of the EO powder at a dose of 600mg/kg body weight along with HFD in the study animals resulted insignificant decrease in the concentration of all antioxidant enzymes and lipid parameters except HDL. The level of HDL increased (P< 0.05) in the EO powder fed rabbits. Moreover, the chances for atherosclerosis also increased with the HFD as shown in Figure 1b. *Emblica officinalis* powder and atorvastatin decrease the atherogenic index and atherosclerosis.

Table1. Comparative lipid profile of different experimental groups and effects of EO powder and atorvastatin on lipid profile after eight weeks of experimentation.

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>Total Cholesterol (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Atherogenic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>105.79±3.37</td>
<td>63.32±2.34</td>
<td>34.19±2.45</td>
<td>72.54±1.98</td>
<td>1.80±0.12</td>
</tr>
<tr>
<td>Group 2</td>
<td>98.61±1.29</td>
<td>44.28±1.13</td>
<td>45.86±1.40</td>
<td>61.28±2.55</td>
<td>0.63±0.07</td>
</tr>
<tr>
<td>Group 3</td>
<td>271.38±5.88</td>
<td>287.30±4.65</td>
<td>17.93±1.84</td>
<td>227.65±3.79</td>
<td>17.50±1.20</td>
</tr>
<tr>
<td>Group 4</td>
<td>127.47±3.24</td>
<td>67.72±1.66</td>
<td>29.28±1.39</td>
<td>88.91±3.12</td>
<td>2.54±0.03</td>
</tr>
<tr>
<td>Group 5</td>
<td>91.48±3.57</td>
<td>39.59±1.23</td>
<td>54.49±1.33</td>
<td>59.76±2.19</td>
<td>1.68±0.34</td>
</tr>
</tbody>
</table>

* P<0.05 when compared the normal group to group II & III.
* P<0.05 when compared the normal group to group II & III.
* P<0.05 when compared the normal group to group II & III.
Table 2. Concentration of antioxidant enzymes in liver and kidneys of various experimental groups after eight weeks of experimentation

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>SOD (mg⁻¹)</th>
<th>CAT (mg⁻¹)</th>
<th>GSH (m mol 100 g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
<td>Liver</td>
</tr>
<tr>
<td>Group 1</td>
<td>10.24±0.78</td>
<td>17.08±0.23</td>
<td>512±3.46</td>
</tr>
<tr>
<td>Group 2</td>
<td>10.89±0.12</td>
<td>18.93±0.31</td>
<td>529±7.21</td>
</tr>
<tr>
<td>Group 3</td>
<td>06.59±0.67</td>
<td>10.03±0.19</td>
<td>442±8.36</td>
</tr>
<tr>
<td>Group 4</td>
<td>10.40±0.47</td>
<td>17.93±0.27</td>
<td>522±9.15</td>
</tr>
<tr>
<td>Group 5</td>
<td>08.99±0.76</td>
<td>16.28±0.91</td>
<td>442±8.36</td>
</tr>
</tbody>
</table>

a: P<0.05 when compared the hyperlipidemic group to the normal group;
b: P<0.05 when compared the EO group to hyperlipidemic group;
c: P<0.05 when compared the statin group to the normal group.

Figure 1a: Mean serum lipid parameters in five groups of experimental rabbits receiving different treatments at the end of 8 week of experimentation.

Figure 1b: Mean atherogenic index in five groups of experimental rabbits receiving different treatments at the end of 8 weeks of experimentation.
DISCUSSION

Rossouw (2015) reported similar findings as observed in the present study, indicating that the intake of HFD is directly proportional to the cholesterol level in the circulating blood. Further, free radicals are produced by HFD leading to hyperlipidaemia and oxidative stress. Lipid peroxidation of high fats leads to high serum cholesterol levels (Anjum et al., 2014). According to Shyamala et al. (2003) the abnormal lipid profile is created by the free radicals produced by the HFD. These free radicals create an oxidative stress leading to the abnormal lipid profile as observed in the current study. It is considered that high TG and TC levels were due to these free radicals. Oxidative stress leads to lipid peroxidation which is the one cause of many diseases, such as diabetes mellitus and coronary heart diseases. Higher LDL and TG levels are the core cause of atherosclerosis, insulin resistance and glucose resistance (Ghuffar et al., 2014).

Administration of EO powder nearly maintained the lipid profile. The anti-hyperlipidemic effects of EO powder are quite comparable with the standard drug (atorvastatin). EO powder may have achieved the results because it is already known that it possesses anti-cholesterol properties (Kumar et al., 2012b). This powder contains various chemicals like, flavonoids, polyphenolic compounds, and dietary fibres, etc., all of which have been reported as anti-hyperlipidemic compounds. The mechanism as to how the level of TG, TC and LDL decreased is not clear but several studies indicate that that EO is rich in flavonoids and polyphenols which are natural antioxidants (Ghuffar et al., 2014; Sairam et al., 2002). These natural antioxidants perhaps stop the lipid peroxidation of fats resulting in the decreased levels of LDL, TG and total cholesterols in the blood (Scartezzini et al., 2006).

Previously, it has been proven that high fat intake significantly alters the antioxidant defence mechanism against lipid peroxidation in animals (Çelik et al., 2017). It has been reported that hyper-lipidaemia blocks the antioxidant defence system by reducing the activity of catalase and SOD which in turn leads to increased lipid peroxide content thus consequently toxic intermediates are produced. Furthermore, HDL results in the decreased concentrations of tissue glutathione level (Lennicke et al., 2017). When experimental rabbits were treated with EO powder containing flavonoids, the glutathione levels was improved. Rutin (natural flavonoids) raised the level of glutathione activity in the tissues of mice when gastric lesions were induced in them (Ganeshpurkar and Saluja 2017). Studies reveal that flavonoids stimulate antioxidative enzymes to cope the oxidative stress (Semiz et al., 2017).

Higher intake of fats creates an oxidative stress inside the body which triggers several pathways/mechanisms for the lipid peroxidation. Cu$^{2+}$-induced LDL oxidation, membrane lipid peroxidation and ADPFe$^{3+}$/NADPH-induced lipid peroxidation are the most common pathways which are altered by the oxidative stress to create hyperlipidaemia. Antioxidant compounds like ascorbic acid, emblicanins, corilagin, ellagic acid and methyl gallate, etc., present in EO powder inhibit these pathways and directly regulate the abnormal lipid production (Asgary et al., 2013). Ascorbic acid and other antioxidant compounds present in EO are also responsible for downregulation of 3 hydroxy 3 methyl-glutaryl coenzyme A reductase activity resulting in the increased biliary cholesterol excretion thus inhibiting the cholesterol absorption through intestine (Hirose et al., 1991). It is also considered that EO powder reduces the high lipids in the body by producing some antioxidant enzymes like glutathione peroxidase, superoxide dismutase and catalases etc., as these have been reported to be produced by other herbs (Cinnamomum verum, Syzygium aromaticum and Amomma subulatum) when administrated to HFD rats (Dhuley, 1999; Shyamala et al., 2003). Since the antioxidant ingredients from plants are helpful against tissue damage, heart disease and cancer, and have now attracted the attention of scientific community and food manufacturers, therefore, the future belongs to functional foods that have beneficial health impacts (Coman et al., 2017).

REFERENCES


