ANTIOXIDANT EFFECT OF FENUGREEK (TRIGNELLA FOENUM GRAECUM) AND TURMERIC (CURCUMA LONGA) IN DIET INDUCED HYPERLIPIDEMIC RATS

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Abstract: The present study was performed to evaluate the possible antioxidant potentials of Fenugreek seeds and Turmeric rhizome on diet induced hyperlipidemic rats. Hyperlipidemic rat models were created by gavaging the rats with high fat diet made up dalda (vanaspathy) and coconut oil at the dose rate of 10 ml/kg body weight for four weeks. Hyperlipidemic rats were thereafter kept on normal diet and gavaged with Fenugreek seeds (2 to 4 g/kg body weight) and turmeric rhizome (200 to 400 mg/kg body weight) powder for four weeks. Hyperlipidemic rats fed with normal diet were used as positive control. It was observed that treatments significantly inhibited levels of TBARS in hyperlipidemic rats. In addition, the activities of catalase, superoxide dismutase and glutathione peroxidase were augmented after herbal supplement. Histopathological examination of the liver and kidney showed fewer lesions in the hyperlipidemic rats treated with herbs compared with control hyperlipidemic rats. The results suggest that fenugreek seeds and turmeric rhizome would be effective in improving the antioxidant levels.

Key words: Fenugreek, Turmeric, Hyperlipidemia,

INTRODUCTION

The peroxidation of lipids containing polyunsaturated fatty acids leads to generation of free radicals that damage tissues and cause disease [1]. To overcome this, the physiological mechanisms such as enzymatic antioxidant defense systems like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and non-enzymatic defense systems like ascorbic acid, alpha-tocopherol and glutathione during hyperlipidemia shall play their vital roles. The direct relationship between hypercholesterolemia and increased production of reactive oxygen species has been established. The antioxidant defense systems are much activated subsequent to increased levels of reactive oxygen species. The antioxidant defense systems represent the essential defense against the potential toxicity during oxidative conditions such as chronic inflammation and abnormal metabolism.

Some naturally occurring compounds or herbs with hypolipidemic and antioxidative potential have beneficial effects such as prevention of the development of overall disease processes. According to a recent estimate of the World Health Organization (WHO), about 70 to 80 per cent of the world population especially in developing countries relies on traditional medicine, mostly plant drugs for their primary healthcare needs [2]. Medicinal plants represent a great source of untapped reservoir of phytochemicals that can be used as drugs. Also, there is a growing interest in the utilization of phytochemicals as phytoceuticals or nutraceuticals.
which are necessary for physiological well being [3]. The scientists, researching on natural products are now intensifying the efforts towards scientific evaluation of medicinal plants used in traditional medicine. The scientists, researching on natural products are now intensifying the efforts towards scientific evaluation of medicinal plants used in traditional medicine.

Fenugreek (Trigonella foenum graecum) is one of the oldest medicinal plants, originating in India and Northern Africa. The leaves and seeds are used to prepare extracts or powders for medicinal use. Along with other legumes, this plant is grown in India, Egypt and Middle East. Indian system of medicine, Ayurveda, gives lot of importance to methi [4]. It is widely grown in Rajasthan, Gujarath, Uttar Pradesh and Tamil Nadu. Trigonella foenum graecum has different names in different vernaculars [5].

Turmeric (Curcuma longa) is used as a spice in cooking vegetables, meat and in preparation of pickles in India and other south Asian countries [6]. The rhizome of Curcuma longa named turmeric is a perennial herb widely cultivated in tropical regions of Asia and Central America. Spices are the natural food additives contribute immensely to the taste and flavour of our foods. In several Asian countries, it has been used as a traditional remedy and for the treatment of many diseases [7].

The fenugreek seeds and turmeric rhizome contribute to the benefits of many physiological roles which need to be scientifically validated. Therefore, the present study was designed to evaluate the individual and combined effects of fenugreek seeds and turmeric rhizome on antioxidant property in Wistar albino rats.

**MATERIALS AND METHODS**

**Experimental animals:** A total of sixty Wistar albino male rats procured from Indian Institute of Sciences, Bangalore at one month age weighing from 40 to 45 g were reared for one more month to attain the body weight of 240 to 260 g. They were randomly distributed into five groups (Group I, II, III, IV, and V) consisting of twelve rats in each group. Permission was obtained from Institutional Animal Ethics Committee with No. 14/LPM/IAEC/2008, dated: 03.01.2009, to conduct the experiment. The polypropylene rat cages were used for rearing of the rats under standard laboratory hygienic conditions.

**Feed:** The food pellets (3000 Kcal/Kg, crude protein: 23.10%, crude oil: 3.15%, crude fiber: 12.05%, ash: 7.45% and sand silica: 0.5%) were obtained from M/s Amrut Laboratory Animal feeds, Bangalore. The feed and water was provided ad libitum.

**Preparation of high fat diet:** Commercially available edible Dalda (Vanaspathy) and culinary grade coconut oil were obtained from local market. The high fat diet (HFD) was prepared by homogeneously mixing Dalda and coconut oil in the ratio of 3:2 w/w.

**Induction of hyperlipidemia:** Group I (negative control) was administered as placebo by oral gavaging technique. For the Group II, III, IV and V, in addition to normal diet and water, high fat diet (HFD) made up of mixture of vanaspathy and coconut oil in the ratio of 3:2 w/w was administered by gavaging to induce hyperlipidemia. HFD was gavaged at the dose rate of 10 ml per kg body weight to each animal orally daily for a period of four weeks. At the end of four weeks, the hyperlipidemia was confirmed by estimating the various components of lipid profile such as total cholesterol, triglycerides, LDL cholesterol, VLDL cholesterol and HDL cholesterol in serum.

**Herbal material:** Good quality seeds of Trigonella foenum graecum (fenugreek) and rhizomes of Curcuma longa (turmeric) were obtained from the local market. Fenugreek is commonly known as methi in Hindi and other regional languages in India and whereas, the turmeric is known as haldi. Both the seeds and rhizomes were identified and authenticated at Department of Plant Pathology, GKVK Campus, Bangalore. The seeds and rhizomes were dried at 40 ºC and finely pulverized. The aqueous solutions of 10 % fenugreek seed powder and 1 % turmeric rhizome powder were prepared freshly on every day.

**Herbal treatment:** Once the hyperlipidemia was induced between 0 to 4th week of the experiment and thereafter by confirming the same at the end of 4th week, from the beginning of fifth week to the end of eighth week, the herbal treatment was carried out. Group I (negative control) and Group II (positive control) were administered with distilled water at the dose of 10 ml per kg body weight to each animal orally for a period of four weeks, as placebo by oral gavaging technique in two divided doses, once in the morning and once in the evening. The 10% aqueous solution of fenugreek seeds powder at the dose rate
of 4 g/kg b.w. (Group III) and one per cent aqueous solution of turmeric rhizomes powder at the dose rate of 400 mg/kg b.w. (Group IV) were administered. The combined administration of the aqueous solution of fenugreek seeds powder at 2 g/kg b.w. and turmeric rhizomes powder at 200 mg/kg b.w. was administered to Group V. They were administered by gavaging in two divided doses, once in the morning and once in the evening.

Collection of organs: At the end of the period of induction of hyperlipidemia, considered as 4th week, six rats from each of the groups were sacrificed to collect liver and kidneys for assay of Thiobarbituric Acid Reactive Substances (TBARS), Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx). Also, at the end of the herbal trial, i.e. on 8th week, six rats from each of the five groups were sacrificed to collect liver and kidneys for assay of TBARS, SOD, CAT and GPx.

Tissue preparation: Immediately after sacrificing the animals, the tissues were processed for estimation of activity of antioxidant enzymes as per the method described by Bruce and Baudry [8]. Liver and kidney samples were isolated and washed in ice cold normal saline to remove blood and it was blotted dry and stored at -20 °C for further analysis. Liver was crushed in tissue homogenizer with 0.05 M phosphate buffer (pH 7.4) to make it 10 per cent liver homogenate w/v (1 g of liver tissue crushed in 10 ml of 0.05 M phosphate buffer). This liver homogenate solution was used for the estimation of TBARS. The part of the liver homogenate was centrifuged at 15,000 g for 1 h at 4°C and the supernatant obtained was used for the estimation of superoxide dismutase, catalase and glutathione peroxidase levels. The kidney homogenate was also prepared in similar manner and used for estimation of TBARS and SOD, Catalase and GPx levels.

Estimation of antioxidant enzymes: Superoxide dismutase (EC 1.15.1.1) was determined by the method as described by Marklund and Marklund [9], Catalase (EC 1.11.1.6) was estimated by the method as described by Caliborne [10] and Glutathione peroxidase (EC 1.11.1.6) was determined by the method as described by Rotruck et al. [11].

Statistical analysis: The results were expressed as mean ± SE. The statistical analysis of the variance

Table 1: Mean ± SE Values of Antioxidant enzymes profile and TBARS level at Post-induction of Hyperlipidemia (4th week) for Four Weeks and after Herbal treatment (8th week) for Four Weeks in Liver. ^P< as compared to 4th week value

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Week</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (units/mg protein)</td>
<td>4</td>
<td>6.12±0.23</td>
<td>12.80±0.51</td>
<td>13.11±0.24</td>
<td>12.6±0.11</td>
<td>13.47±0.10</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>5.24±0.11</td>
<td>14.1±0.23</td>
<td>17.21±0.50</td>
<td>17.60±0.51</td>
<td>18.54±0.22</td>
</tr>
<tr>
<td>Catalase (µmol of H2O2/min/mg protein)</td>
<td>4</td>
<td>163.21±4.13</td>
<td>225.80±7.91</td>
<td>221.32±2.81</td>
<td>228.11±3.10</td>
<td>231.14±1.07</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>165.12±3.40</td>
<td>252.11±2.24</td>
<td>280.43±7.01</td>
<td>281.33±5.30</td>
<td>289.12±3.30</td>
</tr>
<tr>
<td>Glutathione peroxidase (units/mg protein)</td>
<td>4</td>
<td>147.12±3.71</td>
<td>196.92±6.20</td>
<td>201.13±2.31</td>
<td>204.32±2.62</td>
<td>202.63±4.01</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>147.40±1.92</td>
<td>217.22±1.40</td>
<td>252.31±6.03</td>
<td>255.12±6.14</td>
<td>261.02±0.1</td>
</tr>
<tr>
<td>TBARS (µmol of MDA/100 g tissue)</td>
<td>4</td>
<td>0.80±0.31</td>
<td>12.62±0.50</td>
<td>11.23±0.14</td>
<td>12.31±0.40</td>
<td>12.49±0.32</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.71±0.02</td>
<td>9.50±0.02</td>
<td>5.71±0.02</td>
<td>6.22±0.01</td>
<td>6.30±0.04</td>
</tr>
</tbody>
</table>

Table 2: Mean ± SE Values of Antioxidant enzymes profile and TBARS level at Post-induction of Hyperlipidemia (4th week) for Four Weeks and after Herbal treatment (8th week) for Four Weeks in Kidney. ^P< as compared to 4th week value

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Week</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (units/mg protein)</td>
<td>4</td>
<td>5.92±0.11</td>
<td>11.3±0.30</td>
<td>11.92±0.14</td>
<td>11.52±0.32</td>
<td>12.10±0.11</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>6.10±0.2</td>
<td>14.32±0.14</td>
<td>18.62±0.51</td>
<td>18.83±0.41</td>
<td>20.11±0.43</td>
</tr>
<tr>
<td>Catalase (µmol of H2O2/min/mg protein)</td>
<td>4</td>
<td>65.6±1.60</td>
<td>94.72±3.21</td>
<td>98.22±2.91</td>
<td>99.40±1.31</td>
<td>95.32±4.10</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>68.20±1.91</td>
<td>113.6±1.80</td>
<td>136.13±3.40</td>
<td>139.22±1.40</td>
<td>152.21±1.8</td>
</tr>
<tr>
<td>Glutathione peroxidase (units/mg protein)</td>
<td>4</td>
<td>129.31±2.02</td>
<td>191.24±6.31</td>
<td>196.22±4.20</td>
<td>195.51±3.80</td>
<td>198.43±4.11</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>128.31±2.04</td>
<td>220.12±5.81</td>
<td>241.22±6.91</td>
<td>247.24±4.09</td>
<td>251.31±6.10</td>
</tr>
<tr>
<td>TBARS (µmol of MDA/100 g tissue)</td>
<td>4</td>
<td>1.21±0.22</td>
<td>1.12±0.03</td>
<td>12.10±0.12</td>
<td>12.41±0.30</td>
<td>12.61±0.62</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1.12±0.03</td>
<td>7.62±0.10</td>
<td>6.51±0.02</td>
<td>6.13±0.01</td>
<td>6.84±0.01</td>
</tr>
</tbody>
</table>
between control and the experimental values was carried out using two-way ANOVA with Duncan’s multiple comparison tests using computerized GraphPad Prism trial Version 5.00.

RESULTS

Antioxidant enzymes profile: The estimated values of antioxidant enzymes such has SOD, CAT and GPx at the 4th week values are the values recorded at post – induction of hyperlipidemia. The 8th week values are the values recorded at the end of the period of herbal treatment to the hyperlipidemic rats of the experiment (Tables 1,2). After herbal supplementation for a period of four weeks, the fenugreek received group, the turmeric received group and the group that received both fenugreek and turmeric showed significantly (P<0.05) elevated levels indicating that the fenugreek, turmeric and the combination of fenugreek and turmeric were more antioxidant compared to turmeric alone.

Thiobarbituric acid reactive substances (TBARS): The estimated value of Thiobarbituric acid reactive substances (TBARS) at the 4th week values are the values recorded at post – induction of hyperlipidemia. The 8th week values are the values recorded at the end of the period of herbal treatment to the hyperlipidemic rats of the experiment (Tables 1,2). After herbal supplementation for a period of four weeks, the fenugreek received group, the turmeric received group and the group that received both fenugreek and turmeric showed significantly (P<0.05) lowered levels indicating that the fenugreek, turmeric and the combination of fenugreek and turmeric were more antioxidant.

DISCUSSION

The antioxidant enzymes, such as SOD, CAT and GPx constitute a mutually supportive team of defense against ROS. They showed enhanced activities in HFD fed rats as compared to controls. This may be due to the innate mechanism of the body to combat oxidative stress of a milder nature by secreting elevated levels of enzymes. It was opined as the herbs have triggered the secretion of antioxidant enzymes which in turn stopped the oxidative damage. The saponins, neutral detergent fiber and flavonoids present in high quantities in fenugreek seeds could be responsible for antioxidant property and reduction of lipid peroxidation in tissues [13,14]. The findings of the present study in group III, that received fenugreek seed powder was in conformity with the earlier findings such as, the appreciable changes in SOD and CAT activities with fenugreek supplements [15], the significantly increased activities of antioxidant enzymes in diabetic rats supplemented with fenugreek leaf powder and Ocimum basilicum [16,17]. The increased activities of SOD, GPx and CAT may be attributed to the presence of flavonoids in fenugreek [18].

The increase in the activities of SOD, CAT and GPx in Group IV, that received turmeric was in agreement with the earlier findings such as, curcumin supplementation enhanced endogenous antioxidant defense components [19] and flavonoids present in herbs may trigger antioxidant enzyme systems [18]. Antioxidant property was attributed to turmeric antioxidant protein (TAP) present in turmeric [20].

The findings of the present study indicated that the supplementation of fenugreek, turmeric and their combination significantly (P<0.05) reduced the levels of lipid peroxidation compared to Group II. The observation in Group III, which received fenugreek seeds, was in agreement with reduced lipid peroxidation following fenugreek seeds supplementation [14] and reduced lipid peroxidation in streptozotocin induced diabetic rats with fenugreek leaves supplementation [16]. The reduction in TBARS levels in Group IV, that received turmeric rhizome powder, was in conformity with Soudamini et al. [21]. Turmerin, a water soluble antioxidant peptide present in turmeric protects DNA [20]. Similarly, the results were in accordance with Kempaiah and Srinivasan [22] who showed reduced lipid peroxides with dietary curcumin, capsaicin and garlic. Ayeshazafir and Naheedbanu [19] showed enhancement of key endogenous antioxidant defense components with curcumin treatment. The reduced levels of TBARS in the present study were in accordance with Kapoor et al. [23] who reported reduced levels of TBARS in heart homogenate and Shah et al. [24] who reported inhibition of lipid peroxidation with curcumin. Also, heat stable antioxidant principle called as turmeric antioxidant protein (TAP) had been isolated in turmeric by Subramaniam et al. [20]. Phenolic and methoxy groups present in curcumin could be attributed to free radical scavenging activity of curcumin [25]. Soudamini et al. [21] showed that
increased peroxides formed in body were reduced by administration of curcumin which may be due to the scavenging of peroxides and other activated oxygen species formed or due to the neutralization of the free radicals.

CONCLUSION

It was concluded that the supplementation of fenugreek seed powder, turmeric rhizome powder and their combination exhibited antioxidant property in Wistar albino rats. Hence they could be used as herbal remedies during altered conditions and also for physiological wellbeing.

REFERENCES