EFFECT OF ASCORBIC ACID ON ESTIMATION OF OXIDATIVE STRESS BY MEASURING THE LEVEL OF GLUTATHIONE IN THE SERUM OF RABBIT’S BLOOD

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INTRODUCTION

Indian spices that provide flavor, color, and aroma to food also possess many therapeutic properties. Ancient Indian texts of Ayurveda, an Indian system of medicine, detailed the medicinal properties of these plants and their therapeutic usage. Recent scientific research has established the presence of many active compounds in these spices that are known to possess specific pharmacological properties. The therapeutic efficacy of these individual spices for specific pharmacological actions has also been established by experimental and clinical studies. The medicinal effects traditionally ascribed to Indian spices are validated by modern pharmacological and experimental techniques, thus providing a scientific rationale to their traditional therapeutic usage. Many plant-derived molecules have shown a promising effect in therapeutics. Many plant-derived molecules have shown a promising effect in therapeutics [1-4]. Spices and herbs are recognized as sources of natural antioxidants and thus play an important role in the chemoprevention of diseases and aging. Among the plants investigated to date, one showing enormous potential is the pepper family otherwise known as Piperaceae [2-6].

In most reports mention that discrete increases in blood levels of this vitamin reduces the risk of death in all conditions. Although there are many functions of vitamin C, his role in health is discussed mostly in relation to its role as an antioxidant and its effects on cancer, blood pressure, immunity, drug metabolism and urinary excretion of hydroxyl proline [6-8]. Those who consume a diet without vegetables and fruits, alcoholism, in older people with limited diets, severely ill patients with chronic stress and in infants fed cow’s milk. Symptoms of scurvy are follicular hyperkeratosis, gingival swelling and inflammation (in gums), bleeding gums, loose teeth, dry mouth and eyes, hair loss and dry skin, among other symptoms that can lead to death. Studies have revealed that increased consumption of grains, fruits and vegetables is associated with reduced risk of diseases [8-10].

KEYWORDS: Vitamin C, GSH, Antioxidants, blood MDA level

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This may be attributed to the presence of natural antioxidants such as vitamin C, tocopherols, carotenoids, polyphenolics and flavonoids which prevent free radical damage. The plant phenolics are commonly present in fruits, vegetables, leaves, nuts, seeds, barks, roots, etc. The antioxidant property of phenolics is mainly due to their redox properties. They act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators [6, 11-13].

Vitamin C (Ascorbic acid) is the most important vitamin in fruits and vegetables. Except human and other primates, most of the phylogenetically higher animals can synthesize vitamin C (L-ascorbate). More than 90% of the vitamin C in human diets is supplied by fruits and vegetables (including potatoes). Vitamin C is defined as the generic term for all compounds exhibiting the biological activity of L-ascorbic acid [12-14].

Ascorbic acid is the principal biologically active form but L-dehydro ascorbic acid, an oxidation product, also exhibits biological activity. Vitamin C is required for the prevention of scurvy and maintenance of healthy skin, gums and blood vessels. It functions in collagen formation, absorption of inorganic iron, reduction of plasma cholesterol level, inhibition of nitrosoamine formation, enhancement of the immune system, and reaction with singlet oxygen and other free radicals. As an antioxidant, it reportedly reduces the risk of atherosclerosis, cardiovascular diseases and some forms of cancer [13-16].

The non-enzymatic antioxidants which act as scavengers are glutathione, vitamin A, vitamin E, and vitamin C [16]. The antioxidants may be of either the natural ones or the synthetic ones. Commonly used synthetic antioxidants are: butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), propyl gallate and tertiary butyl hydroquinone [9].

While the naturally occurring antioxidants like vitamins are a balanced mixture of redox with reduced and oxidized form, the synthetic antioxidants are unbalanced in this respect and they themselves produce harmful free radicals in some cases, emphasizing the importance of the naturally occurring antioxidants over the synthetic ones [11-13].

In the present study, effects of Ascorbic acid on blood Malonaldehyde (MDA) level was evaluated in an attempt to find its anti-peroxidative potential. Plan of the experiment was in an in-vivo system using New Zealand white rabbit (Oryctolagus cuniculus) as an animal of choice. Rabbit was chosen for the work as it is a mammal & having similarity with human beings. It was also selected due to its easy availability & ease of experiment.

Estimation of Oxidative Stress was performed by measuring the level of Glutathione in the serum of rabbit’s blood before and after the administration of Ascorbic Acid. Estimation was made by using a Standard Curve.

**MATERIAL AND METHODS**

Chemicals like 5, 5-Dithiobis(2-Nitrobenzoic Acid) (DTNB) solution, Glutathione, was purchased from Sisco Research Laboratories Pvt. Ltd., Kolkata, India.

**Preparation of standard curve:**

Solutions of various concentration was prepared to calculate the unknown concentration of GSH and the equation of standard curve of GSH was as follows:

\[ A = (0.0058 \times C) + 0.00049 \]

Where A is Absorbance & C is Concentration

\[
\text{Concentration of GSH = } \frac{[A-0.0000085]}{0.0068} \text{ (nano mole/ml)}
\]

**Figure 1: Design of Experimental Methods**

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RESULT & DISCUSSIONS

Table 1: Effect of Ascorbic acid on blood MDA level

<table>
<thead>
<tr>
<th>ANIMAL No</th>
<th>BODY WEIGHT (kg)</th>
<th>ABSORBANCE OF CONTROL</th>
<th>ABSORBANCE OF TEST (Antioxidant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.8</td>
<td>0.606</td>
<td>0.666</td>
</tr>
<tr>
<td>2</td>
<td>1.8</td>
<td>0.406</td>
<td>0.476</td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
<td>0.509</td>
<td>0.553</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>0.655</td>
<td>0.631</td>
</tr>
</tbody>
</table>

Table 2: Blood MDA content in nano mole/ml in animal models

<table>
<thead>
<tr>
<th>ANIMAL No</th>
<th>BODY WEIGHT (kg)</th>
<th>CONCENTRATION OF CONTROL</th>
<th>CONCENTRATION OF TEST (Antioxidant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.8</td>
<td>89.12</td>
<td>97.94</td>
</tr>
<tr>
<td>2</td>
<td>1.8</td>
<td>59.70</td>
<td>69.99</td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
<td>74.85</td>
<td>81.32</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>96.32</td>
<td>92.79</td>
</tr>
</tbody>
</table>

Table 3: Percentage change in blood MDA content of antioxidant treated animals with respect to control

<table>
<thead>
<tr>
<th>AVERAGE</th>
<th>8.03</th>
</tr>
</thead>
<tbody>
<tr>
<td>STANDARD DEVIATION</td>
<td>8.67</td>
</tr>
</tbody>
</table>

Figure 2: Effect of ascorbic acid on blood GSH level

Results of all the experiment performed are shown in tables 1, 2, & 3 & it was graphically presented in figure 1. Table 1 showed the absorbance of different samples. Table 2 represented the various concentration of GSH content in control animals & antioxidant treated animals. Table 3 shows % change in GSH content with respect to control. Ascorbic acid is highly bioavailable and is consequently the most important water soluble antioxidant vitamin in cells, effectively scavenging reactive oxygen species (ROS). When relating the antioxidant activities of fruit juices to health and disease risk, it is important to consider the contribution of ascorbic acid in addition to that of phenolic compounds with antioxidant activity [6].

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From Table 3, it was concluded that after administration of ascorbic acid, the GSH level has increased. This supports the antioxidant role of ascorbic acid. Ascorbic acid is a well-known antioxidant & it has good antioxidant potential. The results presented in figure 1 are also supported the same thing. Though the results clearly supports the antioxidant capacity of ascorbic acid, but in some experiments it was found and reported that ascorbic acid also has pro-oxidant effect. A final conclusion cannot be drawn considering this little volume of work. We can reach in a final conclusion only after repeated experiments using more animal models.

REFERENCES