Evaluation of the Antioxidant Activity of *Amaranthus spinosus* Linn. by Non-Enzymatic Haemoglycosylation

(Penilaian Aktiviti Antioksidan *Amaranthus spinosus* Linn., dengan Haemoglikosilasi Tanpa Enzim)

B.S. ASHOK KUMAR*, K. LAKSHMAN, K.N. JAYA VEAERA, SALEMULLA KHAN, B. MANOJ & V.B. NARAYAN SWAMY

ABSTRACT

*Amaranthus spinosus* Linn. complete plant material was extracted successively by petroleum ether, chloroform, methanol, and water. All the extracts were subjected to in vitro non-enzymatic haemoglycosylation method, i.e. an oxidation reaction. The degree of haemoglycosylation in the presence of different extracts of *Amaranthus spinosus* were measured colorimetrically at 520 nm. The preventive effect of haemoglobin glycosylation at the two concentration; 0.5 and 1 mg/mL was estimated as follows: pet. ether; 13.1%, 16.4%, chloroform; 5.7%, 12%, methanol: 36.91%, 56.07% and aqueous: 22.2%, 31.01 %, respectively. The α-tocopheral (Vitamin E) was used as standard.

Keywords: *Amaranthus spinosus*; chloroform; haemoglobin; haemoglycosylation; vitamin E

INTRODUCTION

Haemo glycosylation refers to the covalent bonding of blood glucose to the red blood cells. Normally, only a small percentage of blood glucose, usually between 4.5 and 6%, is covalently linked to the red blood cells in haemoglobin of the non diabetes population. Glycosylated haemoglobin is recommended for cheking blood sugar control in people who might be pre-diabetic and monitoring blood sugar control in patients with more elevated levels, termed diabetes mellitus. For a single blood sample, it provides far more revealing information on glycemic behavior than a fasting blood sugar value. That being said, fasting blood sugar tests are crucial in making treatment decision. The American diabetes association guidelines are similar to others in advising that the glycosylated haemoglobin test performed at least two times a year in patient with diabetes (American Diabetes Association 2007).

*Amaranthus spinosus* Linn. (Amaranthaceae) commonly known as Spiny amaranth or Pig weed, is an annual or perennial herb, native to tropical America and found throughout India as a weed in cultivated as well as fallow lands (Anon. 1988). Though whole plant is used as laxative (D’ymock 1976; Varier’s 1996), the root are regarded as highly specific for colic by Hindu physicians (Sivarajan & Balachandran 1994) and in Madagascar they are considered as laxative (Kirtikar & Basu 1987). Traditionally boiled leaves and roots of *Amaranthus spinosus* are given to children as laxative. However the drug is also used traditionally as diuretic, antidiabetic, antipyretic, anti-snake venum, antileprotic, and anti-gonorrheal (Kirtikar & Basu 1987; Varier’s 1996). In Malaysia, *Amaranthus spinosus* is used as an expectorant and to relieve breathing in acute bronchitis. Some tribes in India apply *A. spinosus* to induce abortion (Grubben & Denton 2004). The *A. spinosus* is reported for its anti-inflammatory properties (Olumayokun et al. 2004). Effect on hematology (Olufemi et al. 2003), immunomodulatory activity (Tatiya et al. 2007). Antiandrogenic activity (Murgan et al. 1993a), anthelmintic properties (Assiak et al. 2002) and effect on biochemical changes in epididymis (Murgan et al. 1993b).
The main aim of the study was to investigate antioxidant activity of different extracts of *A. spinosus* by *in vitro* non-enzymatic haemo-glycosylation method.

**MATERIALS AND METHODS**

**CHEMICALS**

Haemoglobin (Nice Chemicals Pvt. Ltd., Cochin), glucose and α-tocopherol were procured from Merck, Mumbai. Ascorbic acid and gentamycin were obtained from Biokem internations Pvt. Ltd., Bangalore and Nicholas Piramal India Ltd., Pithampur. All other reagents and solvents used were of analytical grade.

**COLLECTION OF PLANT AND PREPARATION OF EXTRACT**

The fresh plant of *A. spinosus* was collected from B. Kotha Kota, Chittoor district (AP), India, was authenticated by B.K. Venkatesh, Department of Botany, Government First Grade College, Chickballapur, Karnataka (INDIA). A voucher specimen (SKVC11) was deposited in the college herbarium. The leaves were separated from plant, dried and coarsely powdered. The coarse powder (100 g) was successively extracted with solvents (Pet ether, chloroform, methanol and water) by soxhlet apparatus. The different extracts were concentrated to dryness in vacuum.

**PHYTOCHEMICAL SCREENING**

Preliminary phytochemical screening of pet ether, chloroform, methanol and water extracts was carried out. Quantification of flavonoids, phenols and tannins were carried out (Kokate 1986).

**ANTIOXIDANT ACTIVITY STUDY BY NON ENZYMATIC HAEMOGLYCOSYLATION**

The antioxidant activity of different extracts was investigated by estimating the degree of non-enzymatic haemoglobin glycosylation, measured colorimetrically. Haemoglobin, 60 mg/100mL in 0.01 M phosphate buffer (pH 7.4) was incubated in the presence of 2 g/100 mL concentration of glucose for 72 hours in order to find out the best condition for haemoglobin glycosylation. The assay was performed by adding 1 mL of glucose solution, 1 mL of haemoglobin solution and 1 mL of gentamycin (20 mg/100 mL) in 0.01 M phosphate buffer (pH 7.4). The mixture was incubated in dark at room temperature for 72 hours. The degree of glycosylation of haemoglobin in the presence of different concentration of extracts and their absence, were measured colorimetrically at 520 nm (Yadav et al. 2000).

**RESULTS AND DISCUSSION**

**PHYTOCHEMICAL ANALYSIS**

Alkaloids, cardiac glycosides, flavonoids, tannins, amino acids, carbohydrates and phenolic compounds were present in preliminary phytochemical screening.

**ANTIOXIDANT ACTIVITY STUDY BY NON ENZYMATIC HAEMOGLYCOSYLATION**

Percentage inhibition of haemoglobin glycosylation was measured at two concentrations of all the extracts of *A. spinosus* (0.5 and 1 mg/mL) and results are showed in Table 1. Methanol extract showed more antioxidant activity than petroleum ether, chloroform, and aqueous extract. By phytochemical analysis it was found to contain flavonoids, steroids, terpenoids, saponins, anthraquinone glycosides, volatile oils and amino acids. Flavonoids was found to have antioxidant properties (Asgary et al. 1999). Since glycosylation of protein is an oxidation reaction, antioxidants should be able to prevent this reaction. α-tocopherol (vitamin E) was used as a standard antioxidant compound.

**TABLE 1: The antioxidant activity of different extracts of *A. spinosus linn***

<table>
<thead>
<tr>
<th>S.No</th>
<th>Samples</th>
<th>Conc. (mg/ml)</th>
<th>% of Scavenging</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pet.ether</td>
<td>0.5</td>
<td>13.1±0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>16.4±0.41</td>
</tr>
<tr>
<td>2</td>
<td>Chloroform</td>
<td>0.5</td>
<td>5.7±0.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>12.0±0.32</td>
</tr>
<tr>
<td>3</td>
<td>Methanol</td>
<td>0.5</td>
<td>36.19±0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>56.07±0.2</td>
</tr>
<tr>
<td>4</td>
<td>Aqueous</td>
<td>0.5</td>
<td>22.12±0.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>31.01±0.14</td>
</tr>
<tr>
<td>5</td>
<td>α-Tocopherol</td>
<td>0.5</td>
<td>61±0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>86.68±0.16</td>
</tr>
</tbody>
</table>

The activities were compared with those of α-Tocopherol, values are mean ±SEM of three observations.

Glycosylated haemoglobin is a form of haemoglobin used primarily to identify the average plasma glucose concentration over prolonged periods of time. It is formed in a non-enzymatic pathway by haemoglobin normal exposure to high plasma levels of glucose. Glycation of haemoglobin has been implicated in nephropathy and
retinopathy in diabetes mellitus. Monitoring the Hb_A1c in diabetic patients may improve treatment. In the normal 120 day life span of the red blood cell, glucose molecules join haemoglobin, forming glycated haemoglobin. In individuals with poorly controlled diabetes, increase in the quantities of these glycated haemoglobins are noted. The antioxidant activity of the extracts was concentration dependent. *Amaranthus spinosus* contains flavonoids like rutin and quercetin (Ashok Kumar et al. 2008). It has been reported that rutin and quercetin showed the inhibition of haemoglycosylation (Asgary et al. 1999) as maximum 42% and 52%, respectively.

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**REFERENCES**


B.S. Ashok Kumar, B Manoj & V.B. Narayan Swamy
Department of Pharmacognosy
Sri K.V. College of Pharmacy
Chickballapur-562101
Karnataka, India

K. Lakshman
Department of Pharmacognosy
PES College of Pharmacy
Bangalore, Karnataka India

K.N. Jayaveera
Department of Chemistry
Jawaharlal Nehru Technological University of College of Engineering
Anantapur
Andhra Pradesh, India

Saleemulla Khan
Department of Pharmacognosy
Manipal college of pharmaceutical Sciences
Manipal, Karnataka, India

*Corresponding author; email: ashok4vani@gmail.com

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