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RESEARCH ARTICLE

Enhancement of Antioxidant Defense System by UV-Radiation in Fenugreek as a medicinal plant

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Abstract

In the present investigation the antioxidant enzymes' activities and some antioxidant compounds' contents were studied in Fenugreek (*Trigonella foenum graecum*) under the effect of two different UV-doses for various times. The effect of two UV illumination doses (15 and 30 watt) was examined at different time intervals (1, 2, 3, 4 hr.). UV treatment enhanced the activities of antioxidant enzymes like superoxide dismutase (SOD, EC: 1.15.1.1), guaiacol peroxidase (GPX, EC: 1.11.1.7), glutathione reductase (GR, EC: 1.6.4.2), and ascorbate peroxidase (APX, EC: 1.11.1.11). The non-enzymic antioxidant compounds like reduced glutathione (GSH) and ascorbate were also raised. The higher dose 30 watt was more effective than 15 watt. Reactive oxygen species (ROS) formation like superoxide radical and hydroxyl radical is combined with many of morbid operations. Increasing the content of antioxidant defense system either antioxidant enzymes or antioxidant compounds get rid of the negative processes of reactive oxygen species (ROS).

INTRODUCTION

Interacting unstable molecules that have odd number of electrons in their outer shell like oxygen ions and peroxides are called reactive oxygen species (ROS). ROS are important to any biochemical operation and be a major part of aerobic life and metabolism.

During the normal metabolism of oxygen, ROS are created and have serious functions in homeostasis and cell signaling. The level of ROS can increase as a product for ecological stress (e.g., heat exposure). External sources like ionizing radiation can also stimulated the formation of ROS. Cell functions and structures can be damaged due to ROS increase and known as oxidative stress.

Oxidative damage of protein, lipid, DNA and other molecules due to ROS is combined with many of diseases including ischemia reperfusion tissue damage, atherosclerosis, diabetes, cataractogenesis, muscular dystrophy, and neurological disorders such as Alzheimer's disease (**Frilich and Riederer, 1995**).

ROS have been implicated in several ailments such as neurodegenerative diseases, HIV/AIDS, cardiovascular disease, diabetes and cancer (**Halliwell, 2001; Eze, 2006**). ROS associated diseases depend on the balance of the pro-oxidant and the antioxidant concentration.

The generation of free radical, its concentration and the generation site are important for cellular functions of ROS in cancer (**Liou and Storz, 2010**). Since the free radicals are involved in all of these clinical conditions, antioxidants could effectively prevent their occurrence.

The antioxidant potential in plants is refer to presence of phytochemicals such as flavonoid contents (**Cao et al., 1997**). The antioxidant system composed of low molecular weight antioxidants including ascorbate and

reduced glutathione (GSH) as well as several antioxidant enzymes such as ascorbic acid peroxidase (APX), glutathione reductase (GR), guaiacol peroxidase (GPX) and superoxide dismutase (SOD).

The antioxidants terminate the chain reactions resulted from ROS through abstracting free radical derivatives, as well as prevent oxidation reactions. Also, they have several industrial uses as food preservatives, cosmetics and to block rubber recession.

Some artificial antioxidants like butylated hydroxytoluene (BHT) & butylated hydroxyanisole (BHA) are utilized in manufacture of a lot of foods because of greet role in preventing impairment of foods and extending foods' validity (**Hotta et al., 2002**).

The synthetic antioxidants were found to increase the risk of cancer occurrence and liver damage in human (**Ito et al., 1983**; **Namiki, 1990**). The exact mechanism of the harmful effect of synthetic antioxidants is indefinite however it has been proposed that it caused by their toxic effects counter to natural antioxidants (**Bjelakovic et al., 2007**).

The application of natural antioxidants has become more famous as a mean of increasing the food products' expiry, the treatment of human diseases, and slowing down the ageing process (**Wong et al., 2006; Mosquera et al., 2007**).

Our study aimed to research the UV effects on the antioxidant system in Fenugreek to see whether this plant could be used as a natural source for antioxidants against the free radicals causing the various diseases for mankind.

Materials and Methods

Plant material

The experimental plant involved in this investigation was *Trigonella foenum graecum* (Fenugreek, family Fabaceae). Pure seeds were attended from Egyptian Ministry of Agriculture.

Seeds radiation

Seeds of *Trigonella foenum graecum* were exposed to two various doses of UV radiation (15, 30 watt) at various time intervals (1, 2, 3, 4 hr.) using UV system in Nuclear & Radiation Lab., Physics Department, Faculty of Science, Mansoura University.

Growth of Plant

Seeds were germinated according to **El-Shora (2001)**. They were cleaned by keeping in 10% sodium hypochlorite for 24 hr. The seeds were then matured between paper towels, moistened with distilled water in sterilized plastic trays and were covered and incubated in dark at 25^{o}C.

Preparation of enzyme extract

The enzyme extract was equipped with **El-Shora (2001)**. Samples of enzyme analyses were prepared by homogenization of leaves (5g) with a mortar and pestle in 50 mM potassium phosphate buffer (pH 7.0), 0.2 mM EDTA, 0.1 g polyvinylpyrrolidone (PVP), and 10 ml Triton X-100.

Centrifuge the mixture at 12000g for 20 min at 4^{o}C and use the resulting supernatant for immediate determination of enzyme activities.

Estimation of total protein

The protein content was determined by **Bradford (1976)**. Fifty grams of the fresh leaves were grinded in 100 mM phosphate buffer (PH 8.0). Centrifuge the mixture for 30 min at 10.000g. Then, add 1 ml of supernatant to 5 ml of diluted Coomassie Brillant Blue and mix well. Keep mixture in the dark for 15 min and measure the absorption of the protein in the extract at 595 nm spectrophotometrically. The protein concentration was estimated from the curve using bovine serum albumin.

Enzymes assay

SOD activity

The activity of SOD was examined due to **Beauchamp and Fridovich (1971)**. The reaction mixture included 0.24 mM riboflavin, 2.1 mM methionine, 1 ml Triton X-100, 1.72 mM nitroblue tetrazolium chloride (NBT; in 50 mM sodium phosphate buffer, pH 7.8), and 100 ml of enzyme extract. The activity was expressed as units/mg protein. One unit of SOD is the amount of enzyme that causes 50% inhibition in the rate of NBT photoreduction.

GPX activity

GPX activity was assayed according to **Maehly (1955)**. Three ml of 0.02 M phosphate buffer were mixed with 0.2 ml enzyme extract (in 0.05 M, phosphate buffer, pH 6.4), 1ml of 15 mM guaiacol, and 0.02 ml H_2O_2 (88.2 mM). The activity was determined as the change in OD at 475 nm/min/mg protein.

GR activity

GR activity was assayed as the increase in absorbance at 412 nm when 5.5-dithio-bis-2-nitrobenzoic acid (DTNB) is reduced by GSH to produce 2-nitro-5-thiobenzoic acid (TNB) according to **Smith et al. (1988)**. The reaction mixture contained 0.2 M potassium phosphate (pH 7.5), GSSG (1 mM), EDTA (1 mM), DTNB (0.75 mM), NADPH (0.1 mM), and 0.2 ml enzyme extract in a final volume of 3 ml at 25^{o}C.

The reaction was initiated by addition of GSSG to the reaction mixture after 40 min of incubation. GR activity was expressed as µmol NADPH oxidized/min/mg protein following decrease of absorbance at 340 nm.

APX activity

APX activity was determined by the method of **Nakano and Asada (1987)**. The reaction mixture contained 2.5 ml sodium phosphate buffer (pH 7.4, 50 mM), 0.06 mM EDTA, 0.3 mM ascorbate, 300 µl enzyme extract and 200 µl of 2 mM H_2O_2 in a total volume of 3 ml. The decrease in absorbance at 290 nm was recorded at 25°C for 1 min. The enzyme activity was calculated as mM ascorbic acid oxidized/min/mg protein.

Non-enzymic antioxidants assay

GSH content

Total glutathione (reduced GSH + oxidized GSSG), and GSSG contents were determined according to **Anderson (1985)**. The reaction mixture included 0.5 ml of 0.1 M Na-phosphate buffer (PH 7.5), 0.1 ml of 2 mM NADPH, 0.2 ml of 6 mM 5.5-dithio-bis-2-nitrobenzoic acid (DTNB) and 1 ml of glutathione reductase (GR).

The change in absorbance at 412 nm was followed at 25^{o}C. To determine GSSG content, 1 ml of 2-vinyl pyridine was added to the extract. The content of GSH was obtained by subtracting the GSSG content from total glutathione content.

Ascorbate content

Total ascorbate was estimated according to **Cakmak and Marschner (1992)** with some modifications. A sample of 0.5 g of leaves was extracted with 5 ml of 5% meta-phosphoric acid, and centrifuged at 4000 rpm for 20 min. Total ascorbate (AsA + DHAsA) was measured after reduction of DHAsA to AsA with DTT (1.4 dithiothreitol). The reaction mixture contained 0.2 ml of the supernatant, 0.5 ml of 150 mM phosphate buffer (PH 7.4) containing 5 mM EDTA, 0.1 ml of 10 mM DTT and 0.1 ml of 0.5% (w/v) N-ethylmaleimide (NEM) to remove excess DTT. In the reaction mixture, the color was developed after addition of the following reagents: 0.4 ml of 10% trichloro acetic acid (TCA), 0.4 ml of 44% ortho-phosphoric acid, 0.4 ml of 4% (2, 2-bipyridine) in 70% ethyl alcohol, and 0.2 ml of 3% $Fecl_3$. The mixture was then incubated at 40^{o}C for 30 min and the color produced was recorded at 525 nm.

Statistical analysis

All data in this investigation are presented as mean ± SE obtained from three measurements.

Results and Discussion

The plants contain two types of antioxidants called primary and secondary antioxidants that vary in their mechanisms of action **(Lim et al., 2007)**. Primary antioxidants can sweep free radicals and subscribe electrons or hydrogen atoms to make the free radicals more stable. However, secondary antioxidants suppress the formation of radicals and consequently preventing oxidative damage.

The antioxidant enzymes (e.g. SOD, GPX, GR and APX) activities were promoted in the Fenugreek (Figs. 1, 2, 3, and 4 respectively) in response to UV-B. **Kacharava et al. (2009)** reported that UV radiation affected the antioxidant level in the beet root and cabbage leaves. However, prior-treatment before extraction process and stage of leaf maturity affected the antioxidant activity in the guava's leaves (**Nantitanon et al., 2010**).

The tissues of plant have a careful balance between rates of free-radical production and their removal by evolving regulatory mechanisms for controlling the synthesis of antioxidant enzymes in response to different oxidative stimuli.

The results in the present investigation showed that UV illumination at 15 watt and 30 watt enhanced the increase of GSH in Fenugreek (Fig. 5). The reduced glutathione (GSH) is non-protein thiol formed in most plant species. GSH playing great role in maintaining cellular redox status (**Rennenberg, 1980**). The product of GSH oxidation is its disulfide (GSSG). The later can be reduced back to GSH by glutathione reductase (GR) in presence of NADPH (**Ric de Vos et al., 1994**).

Most of glutathione in living cells is maintained in the form of GSH (**Kosower and Kosower, 1978**). GSH is essential for stabilization of several enzymes. Also GSH can act as a substrate for dehydroascorbate and reacts directly with free radicals including hydroxyl radical to alleviate the enzymes inactivation by oxidation of the essential thiol group (**Saeed et al., 2012**).

The total ascorbate content in the present work increased in response to irradiation (Fig. 6), and this increase was dependent on the dose and the time. Increases in ascorbate concentration in fenugreek leaves were accompanied by higher activities of SOD, APX, and GR. In support, high light intensity (**Gillham, 1987**), in chilling treatment (**Schoner, 1990**) led to increase the ascorbate levels as well as the activities of SOD and APX.

In conclusion, this result indicates that leaves of fenugreek have the potential to be a suitable source of various antioxidants.

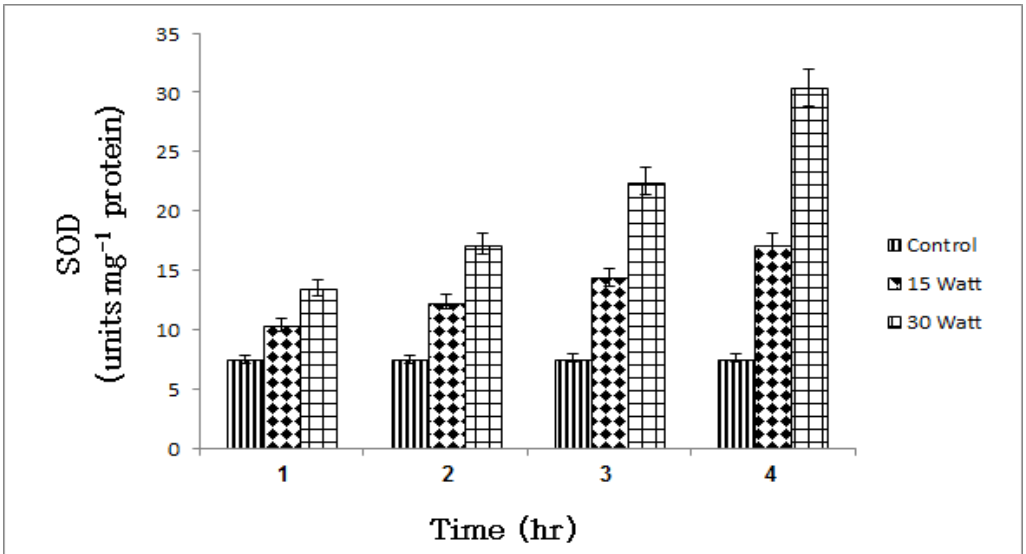

Fig. 1. Effect of UV treatment on superoxide dismutase (SOD) activity

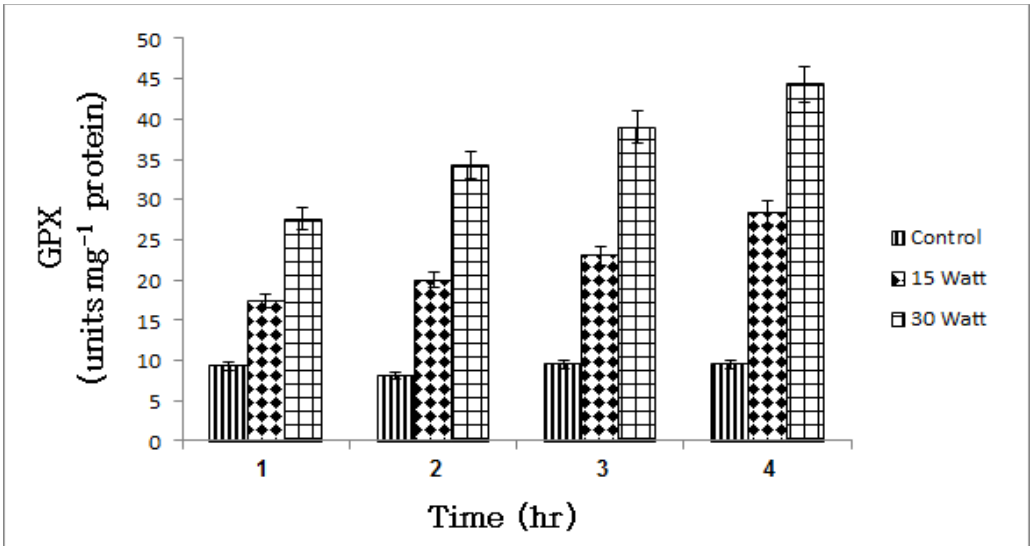

Fig. 2. Effect of UV treatment on guaiacol peroxidase (GPX) activity

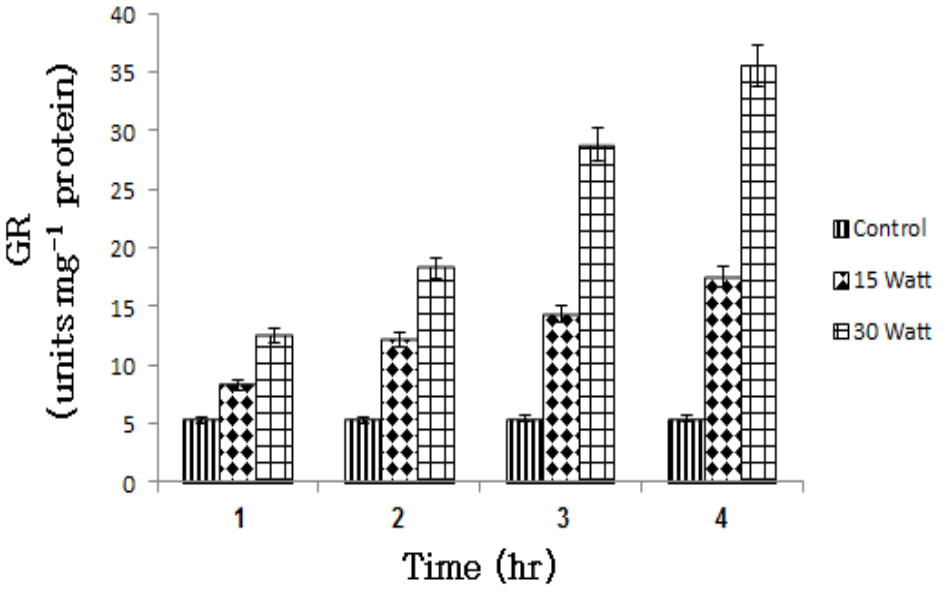

Fig. 3. Effect of UV treatment on glutathione reductase (GR) activity

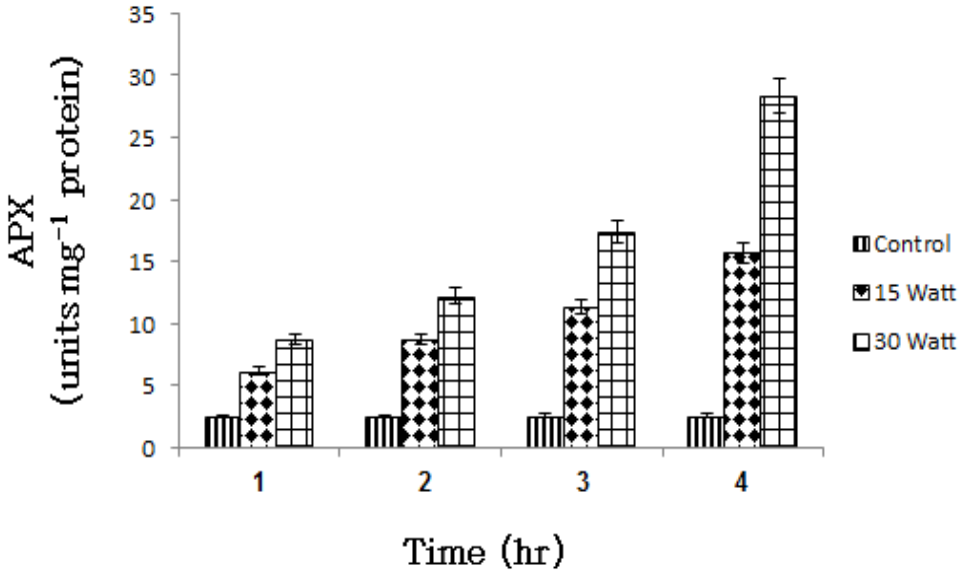

Fig. 4. Effect of UV treatment on ascorbate peroxidase (APX) activity

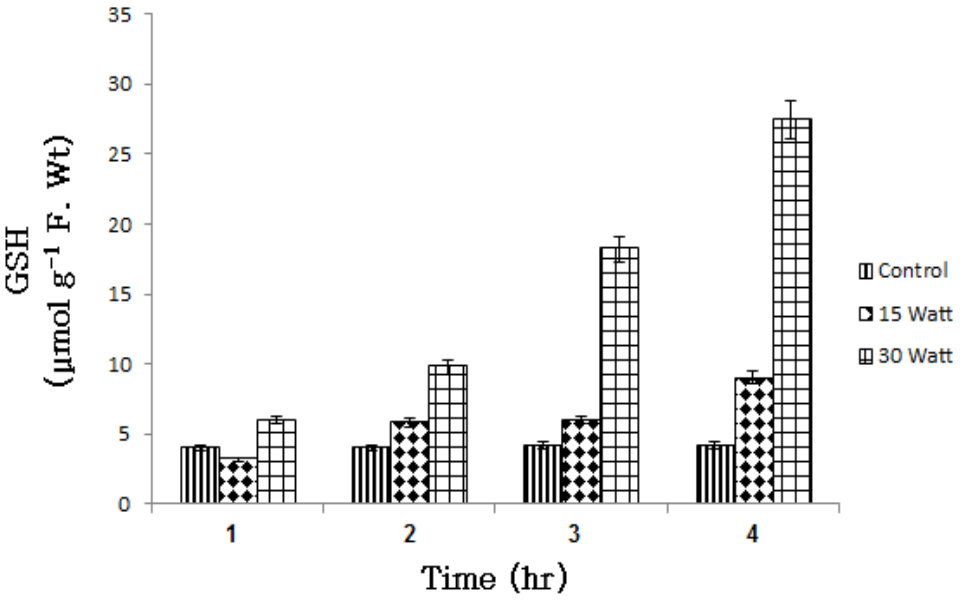

Fig. 5. Effect of UV treatment of reduced glutathione (GSH) content

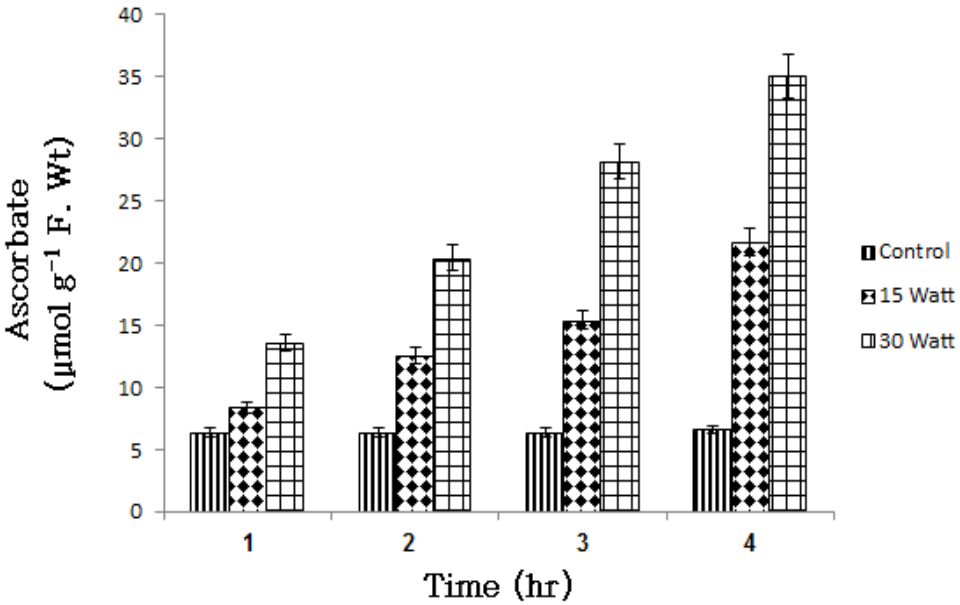

Fig. 6. Effect of UV treatment on ascorbate content

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