Effect of UV-C Radiation on Egyptian Henbane (*Hyoscyamus muticus* L.) Callus Growth and Biochemical Components

Abdelrazik E.[1]; M. A. M. El-hamahmy[2*]; I. Abo-Elsoud[3]; Elham M. Ali[4] and Akram A. Aboseidah[1]
[1]Department of Botany, Faculty of Science, Suez University, Suez, Egypt
[2]Department of Agric. Botany, Faculty of Agriculture, Suez Canal University, Ismailia, 41522, Egypt
[3]Department of Horticulture, Faculty of Agriculture, Suez Canal University, Ismailia, 41522, Egypt
[4]Department of Environmental sciences, Suez University, Suez, Egypt

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Abstract: Physiological impact of different exposure periods (1, 2 and 3 h) of UV-C (253.7 nm) on alkaloids and hyoscyamine production in 21-days aged callus of Egyptian henbane was investigated. Results showed that the concentration of total alkaloids was increased by 2.7 and 2.3 times after exposure to UV-C for 2 or 3 h, respectively more than unstressed callus. Also, data of HPTLC revealed that 2 h exposed-callus accumulated the highest amount of hyoscyamine which similar to the amount in wild leaves but it was more 3.1 times than control. Increment of alkaloids concentration was attributed with high accumulation of non-enzymatic antioxidants as free amino acids, free phenolics and glutathione and increased of peroxidase and superoxide dismutase activity as well as reduction of H_2O_2 concentration. Also, 2 h exposed-callus to UV-C showed high expression of electrophoretic protein bands with molecular weights 109, 42 and 29 KDa. All exposure times to UV-C increased both fresh and dry weight of callus compared to unstressed one. Short exposure time of UV-C enhanced the amount of Chl. a and b in callus than other treatments. All exposure periods of UV-C decreased the concentration of carotenoids, anthocyanine, proline and total carbohydrates than control. Long exposure period of UV-C modified the callus cells shape by induction large spherical cells and lysigenous intercellular spaces. It can be concluded that 2 h exposure time of UV-C is effective treatment for enhancement both alkaloids and hyoscyamine content in callus of Egyptian henbane.

Keywords: Oxidative stress, enzymatic and non-enzymatic antioxidants, medicinal plants, phytochemicals, histology, protein electrophoresis

INTRODUCTION

Egyptian henbane (*Hyoscyamus muticus* L.) is an endemic Solanaceous plants in Egypt, is one of the rich source of alkaloid hyoscyamine (Mahran, 1967). Alkaloids are large nitrogenous compounds establish the defense mechanism that facilitate the interaction with the abiotic stressors and plant (Wang and Wu, 2013).

Ultraviolet (UV) radiations included UV-C (200–280 nm) had a significant biological impact on plants according to radiation dose, exposure time and type of plant tissues (Katerova *et al.,* 2013). UV elicitation is a potential technique for enhancement the secondary metabolites production from different medicinal or other plants. In this respect, elicitation of callus culture by UV increased the canthin-6-one alkaloid by 3.5 times and pyrrolidine by 1.5 times compared to unelicited callus of *Eurycoma longifolia*. Other secondary metabolites such as squalene and 5-(hydroxymethyl)-2-furancarboxaldehyde increased in UV elicited callus of *Eurycoma longifolia* (Parikrama and Esyanti, 2014). Exposure period to UV had also different effect on the types of secondary metabolites. In this respect, 40 min of exposure time to UV (100-280 nm) enhanced the salicylic acid concentration but 30 min of exposure time increased the scopolotein, caffeic acid and syringic acid in *Althaea officinalis in vitro* (Al-obaidi *et al.,* 2014). Also, 10 min of exposure time to UV-C (254 nm) accumulated the highest *trans*-resveratrol concentration in 12 and 15 days-old callus of *Vitis vinifera* (Keskin *et al.*, 2009). However, 5 min of exposure time to UV-C was effective for accumulation of acetyl-11-keto-β-boswellic acid (10-folds) and β-boswellic acid (7 folds) in callus of *Boswellia serrate* (Ghorpade *et al*., 2011). Moreover, long exposure period with low energy such as UV-B (12.6 and 25.3 kJ m^{-2}) had potential effect on flavonoids such as vitexin, isovitexin and apigenin in 7-days callus aged jatropha (*Jatropha curcas*) that similar to or higher than those found in whole leaves (Alvero-Bascos and Ungson, 2012).

High dose or long exposure period of UV-C had deleterious effect on plant cells which accompanied with oxidative stress and production of different types reactive oxygen species (ROS). Plant cells had effective enzymatic systems such as peroxidase, catalase and superoxide dismutase and non-enzymatic compounds such as glutathione and anthocyanin which quench the ROS agents. ROS had deleterious effect on macromolecules function as chlorophylls, enzymes and ribonucleic acids which reduced the plant growth and development (Mittler, 2002). Therefore, re-homeostasis the physiological environment in plant cells under different abiotic stresses such as UV-C, may be enhance synthesizing and accumulation of alkaloids. To achieve that, investigation the effect of different exposure time of UV-C radiation on alkaloids and hyoscyamine production in Egyptian henbane was done.

*Corresponding author e-mail: mhamahmy@yahoo.com

MATERIALS AND METHODS

Seeds preparation and *in vitro* cultivation:

Wild seeds of *Hyoscyamus muticus* L. were collected from Saint Catherine peninsula and identified according to Tackholm (1974). Experiments were carried out at plant physiology and tissue culture laboratory, Botany Department, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. Seeds were dipped in 250 mg l^{-1} of gibberellic acid (GA_3) for 24 h at lab temperature (25±0.5˚C) to break dormancy (Alaghemand *et al.*, 2013). Seeds were washed with tap water and surface-sterilized with 70% ethanol for 2 min, then in 25% commercial bleach (containing 5.25% sodium hypochlorite) with drops of tween-80 for 20 min and finally rinsed 3 times with double distilled sterilized water. The sterilized seeds were cultured in hormone-free MS medium (Murashige and Skoog, 1962), supplemented with 7 g l^{-1} agar and 15 g l^{-1} sucrose and maintained at 25 ± 1°C under light condition (1,500 lux, 16 h/day) for germination. After germination, plantlets have been obtained for explants preparation.

Explant culture and callus induction:

Shoot tip (18 days-old) were excised and cultured on MS medium supplemented with different concentrations of benzyl amino purine (BAP) (0.5 mg l^{-1}) and naphthalene acetic acid (NAA) (0.5, 1.0 and 2.0 mg l^{-1}) for callus induction with 10 replicate. The cultures were incubated at (25±1C°) with (16/8) light/dark cycle. After 4 weeks more suitable callus were selected for further work.

UV treatment:

After four weeks, callus were removed from vessels under aseptic condition and cut a suitable weights (500 mg) and subculture on the same medium for further growth. Callus was irradiated using germicidal sterile lamps (Philips G 36 T6L), 2.5 cm tube diameter, 85 cm length, 41 W and 253.7 nm for 1, 2 and 3 h. The lamps were assembled 45 cm apart and the UV-C field area under the lamps was 60 x 100 cm (Nigro *et al.,* 1998). Callus cultures were incubated at 25 ± 1°C under light condition (1,500 lux, 16 h/day). After 21 days treatment callus was harvested and washed with distilled water to remove remains agar and dried by filter paper.

Vegetative measurements:

Forty pieces of callus fresh (FW) and dry weight (DW) after drying at 70ºC until constant weight was recorded. Water content (%) of callus was calculated using FW and DW values according to Henson *et al.* (1981).

Biochemical determinations:

Photosynthetic pigments (mg 100 g^{-1} FW) in callus (chl. a, b and carotenoids) were determined spectrophotometrically at 662, 644 and 440.5 nm (Arnon*,* 1949). Anthocyanins concentration (mg g^{-1} FW) was determined after digesting callus with 1% of HCl (v/v) in methanol and kept overnight at 4ºC (Lange *et al.,* 1971), then centrifuged at 3000 rpm for 10 min at 5°C. The values are calculated as E_{535}- 0.25 (E_{650})/g FW. Total carbohydrates (mg g^{-1}DW) were determined after digestion by 5 ml of HCl (2.5 N) for 3 hours at 100ºC, then naturalized with sodium carbonate. Sample was made to volume of 10 ml and then filtrated. 50 µl was diluted to 1 ml with distilled water. To each tube, 1 ml of 5% phenol and 5 ml of 96% H_2SO_4 were added. After 10 min, tubes were shaken at 25-30ºC for 20 min. Optical density was measured at 490 nm as reported in Hedge and Hofreiter (1962). Total protein (mg/g FW) was determined by Bradford method (Bradford, 1976) at 595 nm. Proline concentration as mg g^{-1}FW was estimated with ninhydrin reagent as described by Bates *et al.* (1973), the red color intensity was measured at 520 nm against the toluene blank. Total glutathione (µmol/mg protein) was determined at 412 nm by 5-5'-Dithiobis (2-nitrobenzoic acid) (DTNB) reagent (Griffith, 1980). For determination of total free amino acids and free phenols alcohol extraction of callus was prepared as Abdel-Rahman *et al.* (1975). Free phenolics (mg g^{-1} FW) were determined by a modified Folin-Ciocalteu method and measured at 650 nm according to Horwitz *et al.* (1970). Total free amino acids (mg g^{-1} FW) was estimated using the method of Rosen (1957) with ninhydrin reagent. The blue colored were measured against blank sample at 570 nm. Hydrogen peroxide (mmol/g FW) was determined at 390 nm by the modified method according to Shi *et al.* (2007). Malondialdehyde (µmol.g^{-1} FW) determined by the thiobarbituric acid (TBA) reaction as described by Gallego *et al.* (1996). All spectrophotometric analyses were done using UV/VIS spectrophotometer, PG instrument Ltd, USA.

Enzymatic antioxidants activity:

Enzymes extract was prepared according to Urbanek *et al*. (1991). Catalase (CAT, E.C.: 1.11.1.6) activity was determined by measured the oxidation of H_2O_2 at 240 nm (Urbanek *et al*., 1991). The unit of CAT activity was defined as the amount of enzyme, which decomposes 1 mM H_2O_2 per mg^{-1}protein.minute. Peroxidase (POD, E.C.: 1.11.1.7) activity was estimated with 0.1% O-dianisidine and 0.2 M hydrogen peroxide at 430 nm (Urbanek *et al*., 1991). One unit of peroxidase activity was taken as the change of 1.0 unit of optical density per mg^{-1}protein.minute. Superoxide dismutase (SOD, E.C.: 1.15.1.1) activity was assayed by measuring its ability to inhibit reduction of nitro blue tetrazolium at 560 nm as described with (Beauchamp and Fridovich, 1971). One unit of enzyme activity represents the amount of enzyme required for 50 % inhibition of NBT reduction.

SDS-PAGE of soluble proteins:

One dimensional SDS-PAGE gel electrophoresis based on the method of Laemmli (1970) was used to fractionate the soluble proteins in callus. Twenty milligrams of callus were dispersed in 1 ml SDS 10% with 100 µl β-mercaptoethanol for 15 min, then centrifuged at 11000 rpm for 10 min. Twenty µl of extraction were mixed with 20 µl of SDS-loading sample buffer (SDS 4%, β-mercaptoethanol 3%, glycerol 20%, Tris HCl 50 mM pH 6.8 and bromophenol blue traces), heated at 96ºC for 3 min and

10 µl aliquot was electrophoresed (10 µl of protein/lane). The resolving and stacking gels were prepared according to the standard procedure of Davis (1964). The electrode buffer contained 50 mM TRIS, glycine 0.384 M and SDS 0.1%. The protein bands were developed with Commassie Brilliant Blue R-250 dye (0.2% solution, freshly prepared in 45% methanol, 10% glacial acetic acid and 45% distilled water) at room temperature overnight. The gel was photographed and made by scan apparatus as densitometric (optical density) analysis at 600 nm using standard maker protein (Pharmacia).

Histological investigations:

For longitudinal sections (15 µm thick), callus was fixed in formalin acetic acid (FAA), then dehydrated with ethanol series and cleared with ethanol-xylene. Then samples were embedded in paraffin wax at 45-55ºC (Johansen, 1940). Sections were cut with steel blade on rotary microtome. The fixed sections were stained with Safranin O-Fast-green double stain. After staining, sections mounted in Canada balsam (Sass, 1961). Observation and photomicrographs were achieved using research microscope fitted with digital camera Staining Chart (NO.). For staining alkaloid, callus was macerated and stained with Dragendorff's reagent

Alkaloids determination:

To extract alkaloids, powdered dry callus was percolated overnight in methanol till complete exhaustion, then methanol was removed by distillation under pressure. Residue was stirred with 0.1 N HCl and extracted with CH_3Cl. Chloroformic layer was washed with 0.1 N HCl and discarded. The combined acidic layer was then rendered alkaline with NH_4OH and extracted with CH_3Cl. Chloroformic layer was collected, then evaporated, and the final residue was dissolved in desired chloroform (Karawya *et al.*, 1975). Total alkaloids concentration was determined by adding 5 ml of bromocresol green solution (prepared by heating 69.8 mg bromocresol green with 3 ml of 2N NaOH and 5 ml distilled water until completely dissolved and the solution was diluted to 1000 ml with distilled water) and 5 ml of phosphate buffer solution (pH 4.7) (prepared by adjusting the pH of 2M sodium phosphate (71.6 g Na_2HPO_4 in 1 L distilled water) to 4.7 with 0.2 M citric acid (42.02 g citric acid in 1 L distilled water) to a part of chloroform solution. The mixture was shaken and complex extracted with 1, 2, 3 and 4 ml chloroform by vigorous shaking, the extract was then collected in a 10 ml volumetric flask and diluted with chloroform. Atropine standard solution was made by dissolving 1 mg of Atropine (Sigma) in 10 ml distilled water (Ajanal *et al.,* 2012). The absorbance of the complex in chloroform was measured at spectrum of 470 nm in UV-Spectrophotometer against the blank prepared as above but without Atropine.

Determination of Hyoscyamine concentration:

High-Performance Thin-Layer Chromatography (HPTLC**)** was performed on 20 cm × 10 cm HPTLC silica gel 60 F_{254} plates (Merck) with a mobile phase consisting of chloroform : methanol : acetone : aqueous ammonia (25%) 75 : 15 : 10 : 1.6 (v/v/v/v). Hyoscyamine standard (Sigma) was diluted by chloroform to final concentration 20, 40, 60, 80, 120, 160 µg/ml. All samples and standards were applied to the plates by means of CAMAG Linomat 5 with dosing syringe 100 µlas 7 mm bands with 10.5 mm distance between tracks, application X 15 mm and 13 mm application Y edges of plate and the application volume was 1–20 µl for samples and 2-8 µl for standard. Loaded HPTLC plates were developed to a distance of 50 mm in Camag Automatic Developing Chamber CADC 2 at room temperature. The development occurring in a two-steps (preconditioning with 10 ml mobile phase for 5 minutes and development with 25ml mobile phase for 20 minutes). The plate was developed to a distance of 50 mm and dried for 5 min by a stream of warm air, then the plates derivatized with 200 ml Dragendorff's reagent using Chromatogram Immersion Device. Then the plate was scanned and examined densitometry at l = 550 nm by means of CAMAGTLC Scanner 4 with slit dimension of 6 x 0.30 mm.

Statistical analysis:

All data were statistically analyzed as randomized complete blocks design (Steel *et al*., 1997). Analysis of variance (one-way analysis; ANOVA) and means comparisons (Duncan's multiple range tests, 5%) were performed using the MSTAT-C statistical pack-age (M-STAT, 1990).

RESULTS

Effect of UV-C on vegetative growth and total carbohydrates of callus:

All exposure periods of UV-C increased the fresh (FW) and dry (DW) weights of callus compared to control without significant differences among each other (Fig. 1a and b; Fig. 2a). The highest values of FW were detected after 1 and 2 h of exposure time with increment by 40.1 and 34.8% than control, respectively. DW was increased by 1.2 to 1.4 times in UV-C stressed callus than unstressed one. Water content % was insignificantly differed among all treatments (Fig. 1c). Both UV-C exposure periods (1 and 2 h) reduced the total carbohydrates content by 13.3 and 11.1% compared to un-treated callus but long exposure time gave similar concentration with control (Fig. 1d).

Effect of UV-C on callus histology and alkaloids crystal distribution:

3h of UV-C exposure time induced the large irregular spherical cells compared to ovate or oblong shape in other treatments and control (Fig. 2b). The same period gave the highest values of cell length and width as shown in Table (1) with increment by 3.4 and 3.2 times compared to control. Medium and long exposure time of UV-C induced the asymmetry of callus cells with lysigenous intercellular spaces compared to symmetry cells with schizogenous intercellular spaces in low exposure one and control. Abundance of orange–stained alkaloid crystals (Fig. 2c) inside cells were observed in all UV-C treated calli especially that exposed to 2 h of UV-C than control.

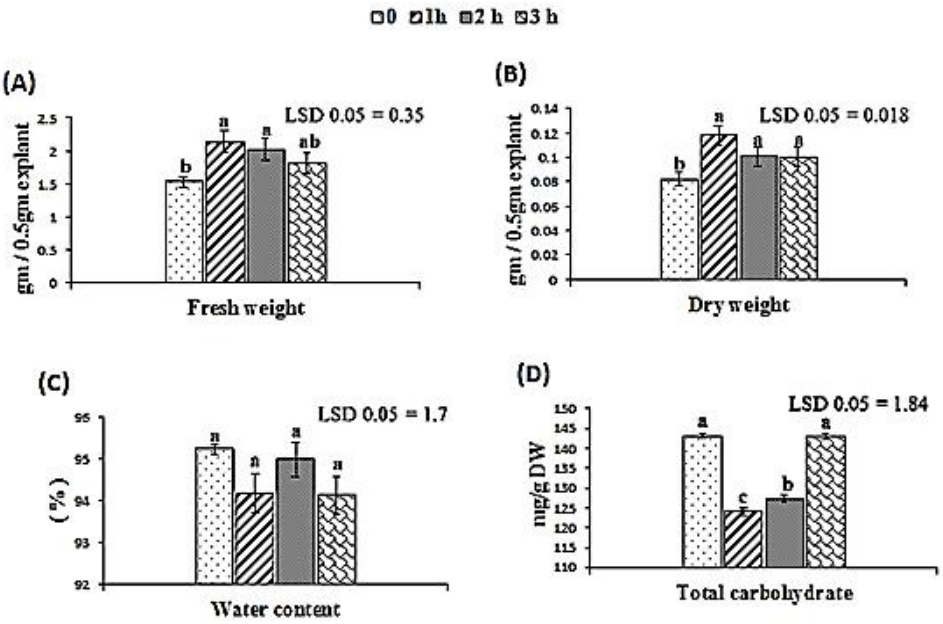

Fig. (1): Effect of different exposure periods of UV-C on fresh, dry weight, water content (%) and total carbohydrate concentration of callus of *H. muticus* L., after 21 days

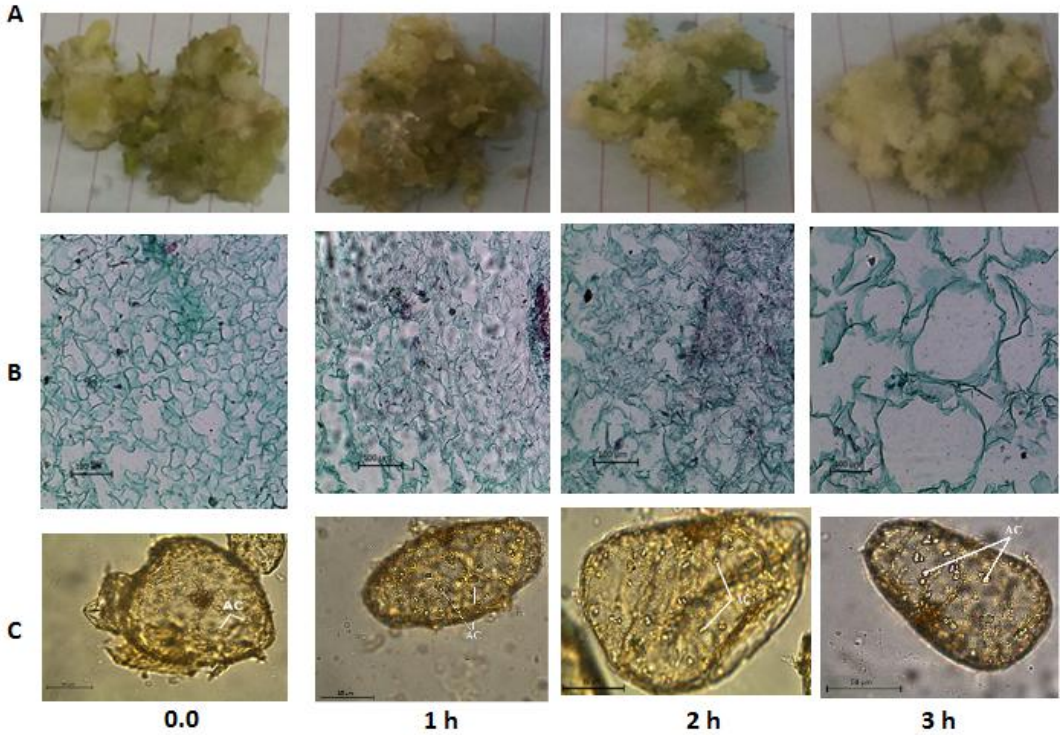

Fig. (2): Effect of different exposure periods of UV-C on growth (A), histology (B) and orange –stained alkaloid crystals distribution (C) in callus of *H. muticus* L., after 21 days; AC (Alkaloid crystals)

Effect of UV-C on pigments:

Short exposure period (1h) had stimulated effect on Chl. a and b content in callus (Fig. 3a and b). The highest concentration of Chl. a and b (3.224 and 1. 815 mg 100^{-1} mg FW, respectively) was determined in 1h exposed-callus with 14.4 and 10.9% of increment than control. Long exposure period of UV-C decreased both Chl. a and b content. Both carotenoid and anthocyanin content were decreased in UV-C stressed-callus at all exposure times than control (Fig. 3c and d). 2h of UV-C exposed callus recorded the highest reduction of carotenoids by 1.8 and 3h of UV-C exposed one gave the highest reduction of anthocyanin by 3.1 times compared to control.

Table (1): Callus cells description after 21 days as affected by different periods of UV-C

UV (h)	Cell			Symmetric of cells	Intercellular space
	Shape	Length µm	Width µm		
0.0 (control)	Regular ovate	72	54	Symmetric	Schizogenous
1	Regular oblong	72.7	50	Symmetric	Schizogenous
2	Irregular oblong	58.2	36.4	Asymmetric	Lysiogenous
3	Irregular spherical	242.8	171.4	Asymmetric	Lysiogenous

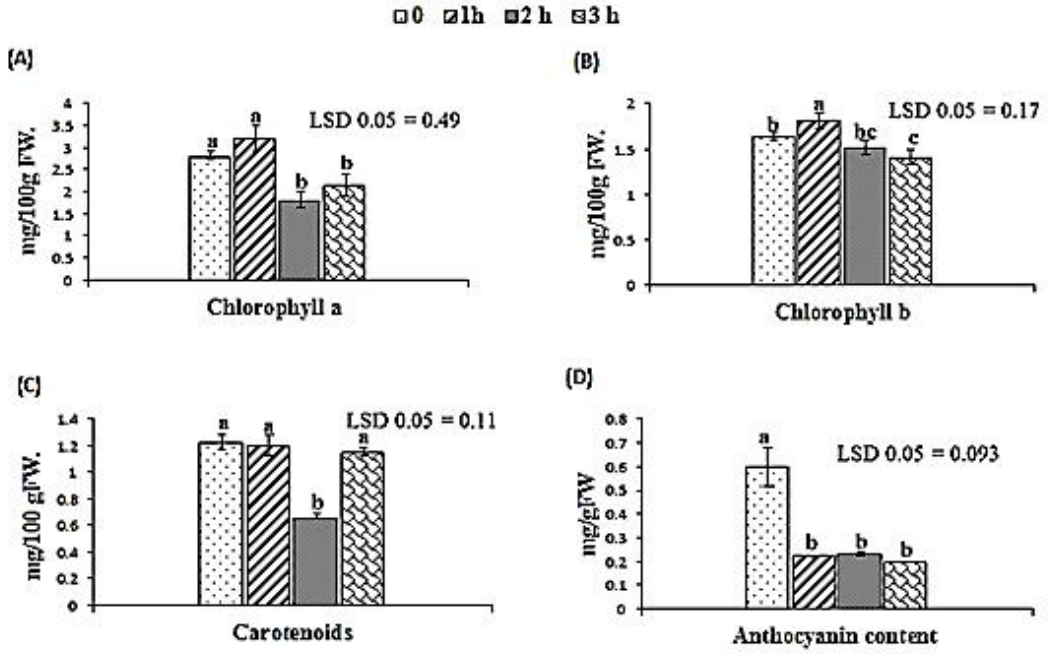

Fig. (3): Effect of different exposure periods of UV-C on pigments (Chl a, Chl b, carotenoid and anthocyanin) concentrations of callus of *H. muticus* L., after 21 days

Effect of UV-C on non-enzymatic antioxidants and H_2O_2:

Glutathione concentration was increased at all exposure times of UV-C compared to control. 2 h of UV-C exposure time induced the highest content of glutathione (4.87 µmol mg^{-1} protein) with increment by 55.1% than control (Fig. 4a). Both 1 and 2 h of UV-C exposure time increased the concentration of free phenolics with increment by 18.5 and 9.7%, respectively compared to control (Fig. 4b). The concentration of proline unchanged among UV-C periods or control (Fig. 4c). The concentration of H_2O_2 decreased in all UV-C treated callus compared to unstressed one. Insignificant differences were detected between UV-C treated callus for 2 and 3 h and untreated one (Fig. 4d).

Effect of UV-C on enzymatic antioxidant activity and malondialdehyde:

2 h exposure period of UV-C induced the highest activity (1.185 unit $100mg^{-1}$ protein min.) of peroxidase (POD) with increment by 2.5 times than control (Fig. 5a). Activity of superoxide dismutase (SOD) was gradually increased by prolonging the exposure time of UV-C. 3 h exposure time of UV-C gave the highest activity of SOD (0.333 unit mg $100g^{-1}$ protein min.) with increment by2.2 time than control (Fig. 5b). Catalase (CAT) activity was reduced in all UV-C treatments compared to control (Fig. 5c). Although long period of UV-C exposure time enhanced the concentration of malondialdehyde (MDA), insignificant differences with other treatments were detected (Fig. 5d).

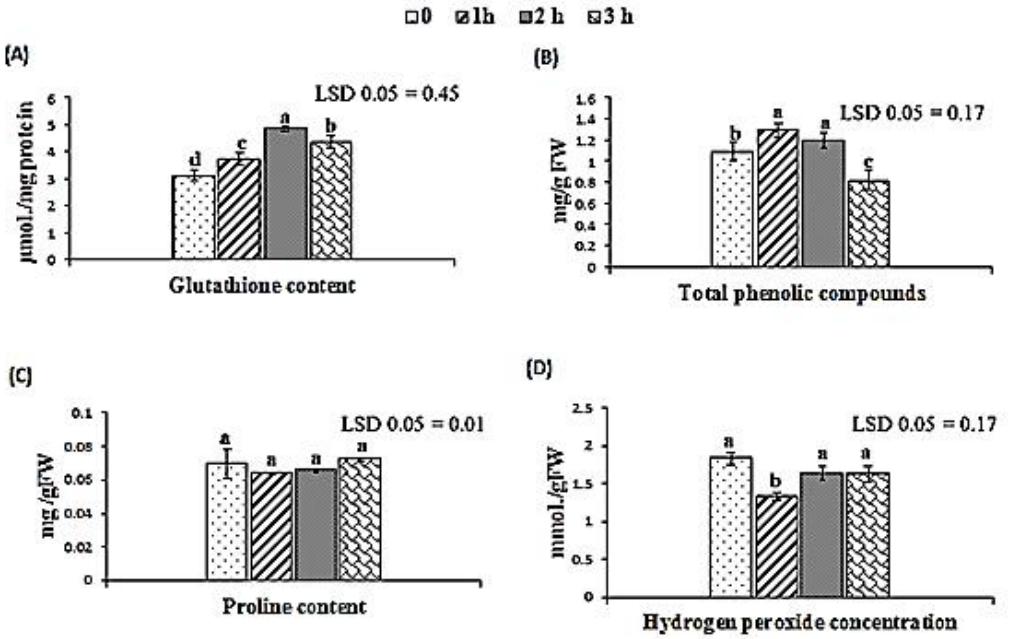

Fig. (4): Effect of different exposure periods of UV-C on glutathione (A), total phenolic compound (B), proline (C), and H_2O_2 (D) concentrations of callus of *H. muticus* L., after 21 days

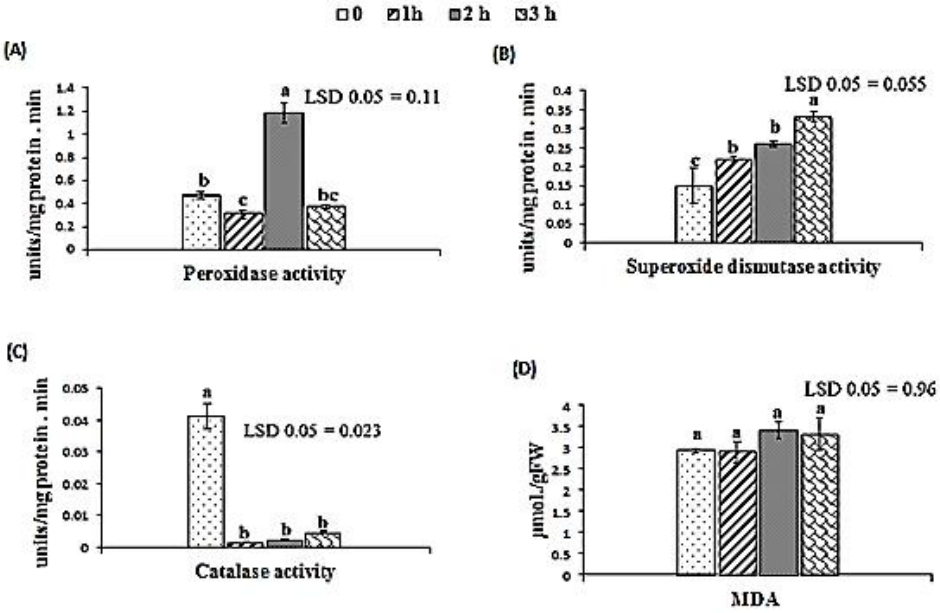

Fig. (5): Effect of different exposure periods of UV-C on POD (A), SOD (B), CAT (C) activity and MDA (D) content of callus of *H. muticus* L., after 21 days

Effect of UV-C on nitrogenous compounds:

Amino acid content was gradually increased with extending of UV-C exposure time. The highest amount of free amino acids (3.04 mg g^{-1} FW) was determined in 3 h of UV-C exposed callus with increment by 94.9% compared to control (Fig. 6a). Protein content was decreased in all UV-C treatments than control (Fig. 6b). The highest reduction (28.8%) was observed in 2 h of UV-C exposed callus compared to control one. Exposure to UV-C for 2 h induced the highest concentration of total alkaloids (44.86 mg g^{-1} DW) with increment by 2.7 time followed by UV-C for 3 h, which induced it by 2.3 time than control (Fig. 6c). Also, 2 h exposed-callus accumulated the highest amount of hyoscyamine (2.523 mg g^{-1} DW) which similar to the amount in wild leaves (2.806 mg g DW) with 3.1 times of increment compared to control (Fig. 6d and Fig. 7).

Effect of UV-C on protein profile:

12 well visible protein bands with different molecular weights from 240 to 10 KDa were observed after protein electrophoresed on polyacrylamide gel (Fig. 8). Unique band with 64 KDa was detected only in 1 h of UV-C exposed callus. Both bands with molecular weight 109 and 69 KDa were appeared in all UV-C treatments and disappeared in unstressed callus. All electrophoretic protein bands had more expression values (as optical densities) than control. High expression values of protein bands with high and low molecular bands 240, 38, 17 and 10 KDa was detected in 1 h of UV-C exposed callus. High expression values of protein bands with medium molecular weight 109, 42 and 29 KDa was detected in 2 h of UV-C exposed callus. Only protein band with molecular weight with 69 KDa had high expression in 3 h of UV-C exposed callus.

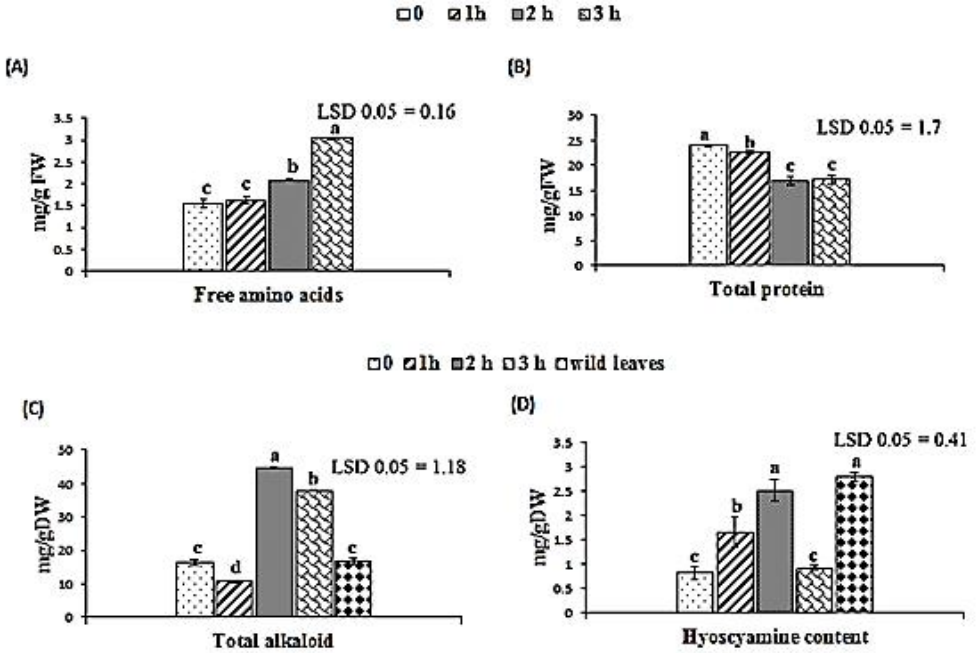

Fig. (6): Effect of different exposure periods of UV-C on free amino acids (A), protein (B), total alkaloid (C), and hyoscyamine (D) concentrations of callus of *H. muticus* L., after 21 days

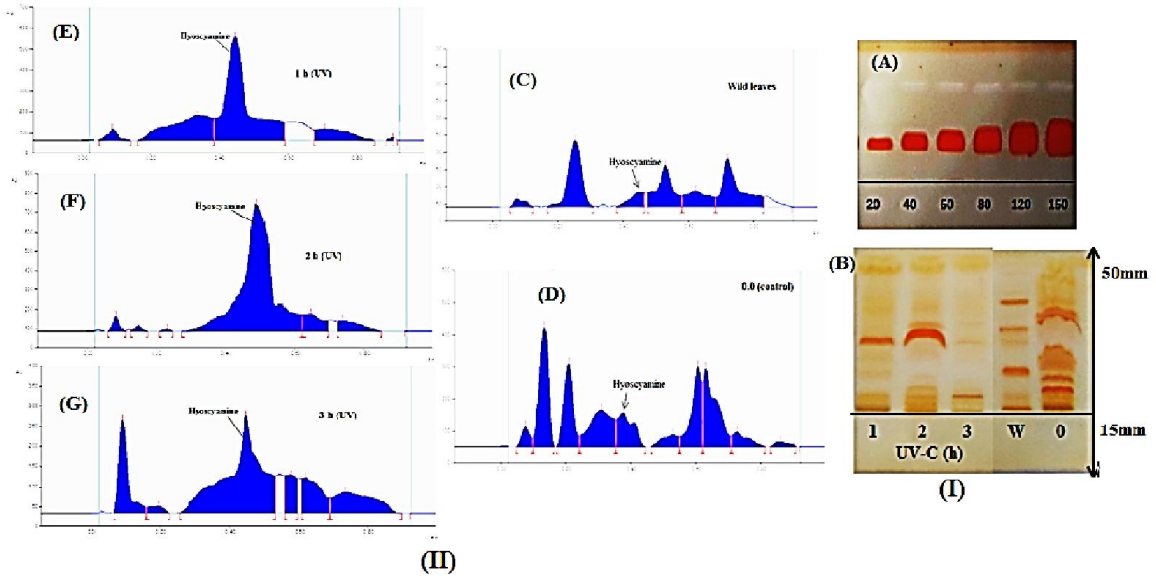

Fig. (7): Chromatogram of alkaloid fraction from (A) hyoscyamine standard solutions (µg/ml) and (B) *H. muticus* callus. (II) Densitogram of alkaloid fraction from *H. muticus* callus recorded at λ= 550 nm after derivatisation with Dragendorff's reagent, (C) wild leaves, (D) unexposed callus extract, (E) 1h UV-C (F) 2h UV-C and (G) 3h UV-C exposed callus

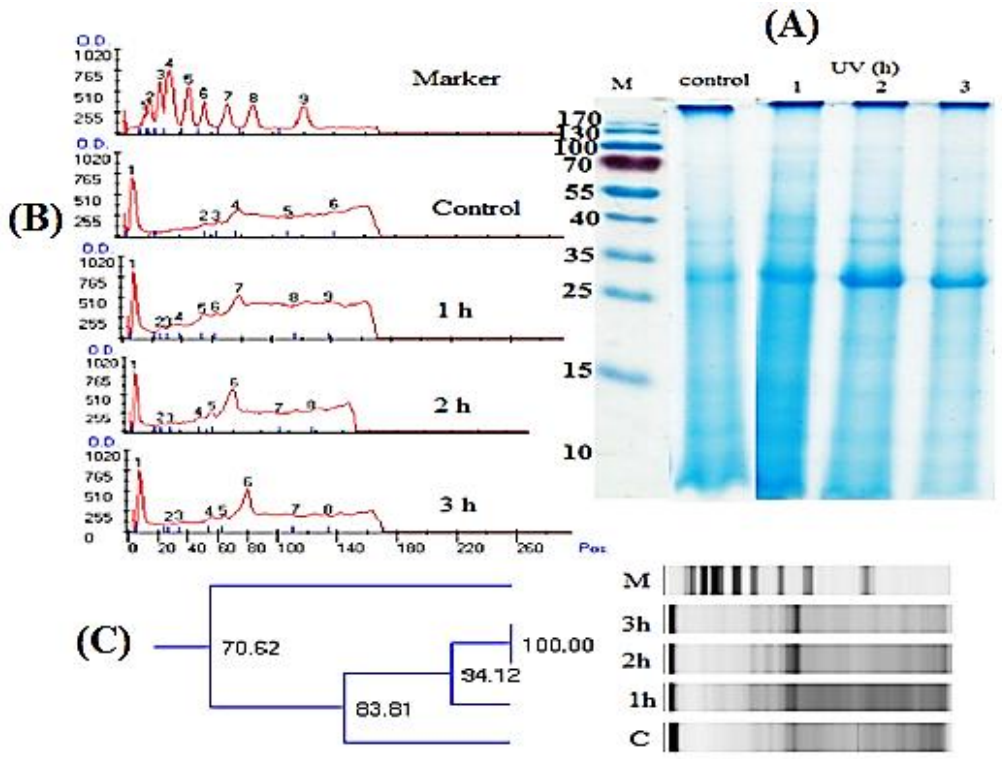

Fig. (8): SDS-PAGE of protein profile (A), optical densities of each protein band (B) and similarity (C) of each exposure period of UV-C of callus of *H. muticus* L., after 21 days

DISCUSSION

UV elicitation is a potential technique to enhance secondary metabolites production as alkaloids and terpenoids in callus of different plants such as *Eurycoma longifolia* (Parikrama and Esyanti, 2014) and grape (Cetin, 2014). Previous literatures reported that low doses of UV-C induced the production of different protective molecules in plant cells such as phytoalexins, pathogensis related proteins (chitinases, glucanase), flavonoides, phenolic acids, lignin, suberin, as well as the antioxidant enzymes such as catalase (CAT), peroxidase (POD) and polyphenyl ammonia lyase (Hopkins and Huner, 2004).

Results reported herein showed that the increment of total alkaloids concentration by 2.7 and 2.3 times after exposure to UV-C for 2 or 3 h, respectively compared to untreated callus as shown in Fig. (6c). Also, Data of HPTLC revealed that, 2 h exposed-callus accumulated the highest amount of hyoscyamine which similar to the amount in wild leaves with 3.1 times of increment compared to control (Fig. 6d and Fig. 7). These increment was attributed with high accumulation of fresh (FW) and dry (DW) weight of callus (Fig. 1a and b; Fig. 2a). Effect of UV on vegetative and physiological parameters of callus differed according to its type and exposure time (Cetin, 2014). This results in line with Manaf *et al.* (2016) who found that long exposure time to UV-B increased all growth parameters in *Echinacea purpurea* callus. However, Al-obaidi *et al.* (2014) showed insignificant increment of both FW and DW of *Althaea officinalis* callus after exposure to UV-C for 30 min while short exposure time reduced both of them. In contrary, long exposure time to UV-C had deleterious effect on biomass of 28 d –old callus of *Eurycoma longifolia* (Parikrama and Esyanti, 2014). Increment of callus FW or DW was not correlated with accumulation of carbohydrates, so UV-C treatment decreased the concentration of carbohydrates as Oraibi (2017) observed in both UV-C treated intact plants or callus of *Moringa oliefera* compared with control.

Exposure to UV-C was accompanied with increment of the concentration of free amino acids in callus. Previous literatures reported that amino acids used as precursors for synthesizing different important compounds in plant such as tryptophan which induced the growth stimulated phytohormone auxine or ornithine which synthesis the cell protective alkaloids (Hopkins and Huner, 2004).

Results demonstrated that Egyptian henbane may be depend on different non-enzymatic antioxidant system as defense mechanism to quench the reactive oxygen species. Exposure to UV-C increased the concentration of glutathione. Therefore, low concentration of H_2O_2 in UV-C stressed callus due to its reduction to water in the chloroplast by glutathione was found (Hopkins and Huner, 2004). The concentration of H_2O_2 (1.3 -1.6 mmol g^{-1} FW) in

Egyptian henban callus was optimum to produce the high amount of alkaloid and normal callus cells. This result coordinated with Zacchini and De Agazio (2004) who reported that highly increment of H_2O_2 content induced a slight cellular damage in both upper and lower layers of *Nicotiana tabacum* callus cultures after 24 and 48 h UV-C treatment.

Increment of phenolics concentration in UV-C stressed callus was detected. This results were in line with Cetin (2014) who found that total phenolics content was increased in calli exposed to UV-C for 5 min after 24 h in grape. Accumulation of soluble phenolic compounds such as lignin accompanied with deposition in cell walls and intercellular space and by deposition of a lignin like material on the surface of UV-B- stressed callus of *Camellia sinensis* (Zagoskina *et al.,* 2003). In contrary, severe and high doses of UV-B decreased the phenolics content, as well as modified the oxidative damage of damiana *in vitro* plants (Soriano-Melgar *et al.*, 2014). The concentration of phenolics differed according to UV-C irradiation distance, irradiation duration and incubation period as Cetin (2014) found in *Vitis vinifera* callus, so calli exposed to UV-C for 5 min with 30 cm distance apart after 24 h had the highest total phenolic content. Also, 2 h of exposure time to UV-B of *Echinacea purpurea* callus increased the total phenolics and the maximum increase in cell suspension was achieved by 4 h (Manaf *et al.*, 2016).

Results reported herein revealed that proline content unchanged among all UV-C stressed callus or unstressed one. Result was disagreed with Oraibi (2017) who found that UV-B for 30 min was increased the proline concentration in *Moringa oliefera* callus compared to the control. However, UV-C treatment decreased the concentration of anthocyanin which contrary to Khatami and Ghanati (2011) who reported the significant increment of anthocyanin content in callus of *Malva neglecta* after UV irradiation compared with the control.

Exposure to UV-C increased the activity of both enzymatic antioxidant peroxidase (POD) and superoxide dismutase (SOD). These findings were in line with Soriano-Melgar *et al.* (2014) who cleared that SOD activity was increased after exposure to UV-B radiation for 2 or 4 h in damiana *in vitro* plants but POD activity had no significant differences with control. Also, Zacchini and Agazio (2004) showed that ascorbate peroxidase and glutathione reductase activity activities was increased in *Nicotiana tabacum* callus after UV-C irradiation. While, catalase (CAT) activity did not change. Also,2 and 4 h exposure time of UV-B increased POD activity in *Echinacea purpurea* callus (Manaf *et al.*, 2016). CAT activity was elevated in grapevine callus cultures after exposure to UV-B (Steel and Greer, 2005).

High expression of protein bands with molecular weights 109, 42 and 29 KDa was found in 2 h exposed callus. This result was agreed with Parikrama and Esyanti (2014) who found highly expression of protein bands with molecular weight 37.5, 40, 50, 51 kDa that participate in synthesis of secondary metabolites responsible for farnesyl diphosphate (FDP) synthase enzyme, strictosidine synthase, geranyldiphosphate synthase, and ornithine decarboxylase, respectively.

CONCLUSION

Elicitation callus of Egyptian henbane with UV-C for 2 h was effective for accumulated the highest amount of hyosyamine. Callus under UV-C treatment enhanced the free amino acids, free phenolics and glutathione content as well as increased the peroxidase and superoxide dismutase activity. UV-C treatment increased both fresh weight and dry weight with enhanced the amount of photosynthetic pigments.

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تأثير الأشعة فوق البنفسجية على نمو الكالس والمركبات البيوكيميائية في نبات السكران المصري

إيمان عبد الرازق محمد[١]، محمد علي الحماحمي[٢]، إسلام أبوالسعود[٣]، إلهام محمود علي[٤]، أكرم عبد الرحمن أبوسعده[١]

[١] قسم النبات - كليه العلوم - جامعه السويس – الإسماعيلية - مصر

[٢] قسم النبات الزراعي - كليه الزراعة - جامعة قناة السويس - الإسماعيلية - مصر

[٣] قسم البساتين - كليه الزراعة - جامعة قناة السويس – الإسماعيلية - مصر

[٤] قسم العلوم البيئية - كليه العلوم - جامعه السويس – السويس - مصر

تم دراسة التأثير الفسيولوجي لفترات التعرض للأشعة فوق البنفسجية (ساعة - ساعتين - ثلاث ساعات) علي إنتاج القلويدات الكلية والهيوسيامين في كالس السكران المصري بعمر ٢١ يوم. أوضحت النتائج زيادة تركيز القلويدات الكلية بمعدل ٢.٧ ، ٢.٣ مره بعد التعرض لمده ساعتين وثلاث ساعات من الفترات علي التوالي مقارنه بالكنترول. أوضحت نتائج التحليل الكروماتوجرافي (HPTLC) زيادة الهيوسيامين بعد التعرض لفترة ساعتين لدرجه مساوية لتركيزه في الأوراق البرية ولكن بزيادة مقدارها ٣.١ مره عن الكالس غير المعرض للأشعة. ارتبطت زيادة القلويدات بارتفاع محتوي الكالس من المركبات المضادة للأكسدة الأنزيمية وغير الأنزيمية مثل الأحماض الأمينية الحرة والفينولات الحرة والجلوتاثيون كما ازداد نشاط كلا من البروكسيديز والسوبر أوكسيد ديسميوتيز بالإضافة إلي انخفاض تركيز فوق أكسيد الهيدروجين كما أن التعرض لفترة ساعتين للأشعة فوق البنفسجية أدي إلي زيادة تعبير البروتينات ذات الوزن الجزيئي ١٠٩- ٤٢ – ٢٩ كيلودالتون. جميع فترات التعرض للأشعة فوق البنفسجية أدت إلي زيادة الوزن الطازج والوزن الجاف للكالس مقارنة بالكنترول. التعرض لفترة قصيرة من الأشعة فوق البنفسجية (ساعة) أدي لزيادة تركيز الكلوروفيل أ و ب مقارنه بباقي المعاملات. كما أدت جميع فترات التعرض للأشعة فوق البنفسجية إلي نقص تركيز الكاروتينيدات والأنثوسيانين والبرولين والكربوهيدرات الكلية مقارنة بالكنترول. التعرض لفترات طويلة للأشعة فوق البنفسجية أدت لتحورات نسيجية في الكالس حيث أدت لزيادة استدارة وكبر حجم الخلايا وظهور المسافات البينية الإنقراضية بين الخلايا. يمكن استنتاج أن تعريض كالس السكران المصري للأشعة فوق البنفسجية لمدة ساعتين تعتبر معامله فعاله في زيادة إنتاج كلا من القلويدات الكلية والهيوسيامين.