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Biochemical changes in heavy metal toxicity induced *Withania somnifera* (L.) Dunal plants

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Abstract

Withania somnifera (L.) Dunal (Solanaceae) is one of the well-known medicinal plants commonly called as Ashwagandha and also known as “Indian ginseng”. It contains Withanolides which are biologically active secondary metabolites, Anahygrine, Anaferine, sominiferine, sominiferinine, withanine and withananine are also present in roots and leaves. *Withania somnifera* (L.) Dunal roots, leaves and preparations of the whole plants are traditionally used in Ayurveda and Unnani for various ailments. It is also having a high medicinal value and possesses potent anti-tumor and antioxidant properties. The present study deals with pot culture experiments with plants were grown in different treatments in black soil, Control (C): a control without any heavy metal treatment to the soil, Treatment No I: Cadmium 10ppm, Chromium 20ppm, Nickel 16ppm were introduced into the soil, Treatment No II: one % of Calcium hydroxide was also added along with heavy metals to soil. Then plants were grown up to the productivity levels. The heavy metal treated plants (Treatment No. I) showed lowest contents of soluble protein, non-reducing sugar, RNA, DNA, phenol, free proline, catalase and peroxides activity when compared to other treatments. The heavy metal treated plants (Treatment No. I) supported increase of total sugar, reducing sugar and polyphenol oxidase activity when compared to control plants and other treatment.

Keywords: *Withania somnifera* (L.) Dunal, heavy metals, biochemical changes

Introduction

Withania somnifera (L.) Dunal is an important medicinal plant, belongs to the family Solanaceae, commonly known as ashwagandha or winter cherry, is used in more than 100 formulations of Ayurveda, Unani and Siddha and is therapeutically equivalent to ginseng (Sangwan *et al.*, 2004) [28]. It is well known for its rejuvenating properties, and hence called “Indian Ginseng” (Singh and Kumar, 1998) [32]. It contains Withanolides which are biologically active secondary metabolites, Anahygrine, Anaferine, sominiferine, sominiferinine, withanine and withananine are also present in roots and leaves. *Withania somnifera* (L.) Dunal roots, leaves and preparations of the whole plants are traditionally used in Ayurveda and Unnani for various ailments. It is also having a high medicinal value and possesses potent anti-tumor and antioxidant properties. Especially in plant tissues, up to 80% of iron and heavy metal is found in the chloroplasts (Hansch and Mendel, 2009) [12]. But iron and heavy metal is toxic when it accumulates to high levels. It can act catalytically via the Fenton reaction to generate hydroxyl radicals, which can damage lipids, proteins and DNA, all of which could lead to growth inhibition (Li, *et al.*, 2012) [18]. The actions of peroxidase (POD) and polyphenol oxidase (PPO) enzymes primarily affect the ability of fresh and processed fruits and vegetables to retain their characteristic flavor and color (Burnette, 1977; Vamos-Vigyazo, 1981) [5, 37]. Total phenol and flavonoid contents of fresh-cut and treated cantaloupe were determined by a modification of the method (Amerine and Ough, 1980) [1].

The stress effects on plants include changes in leaf pigments, altered physiology (impaired photosynthesis), ultimately poor growth, less vigor, and sometimes even death (Larcher, 1995) [16]. The yield reduction is mediated through reduced leaf growth and consequently lower photosynthetic productivity (Chen, *et al.*, 1993) [7]. Drought affects nearly all the plant growth processes depending on the intensity, rate and duration of exposure and the stage of crop growth (Brar, *et al.*, 1990) [2]. Proteins are important constituents of the cell that are easily damage in environmental stress condition (Prasad, 2002; Wu, *et al.*, 2005; Chen, *et al.*, 2000;

Singh, *et al.*, 2007) [8, 25, 33]. The phytochemical activity at PS2 and electron requirement for photosynthesis, and ultimately to increased susceptibility to photo damage (Flagella, *et al.*, 1998) [10].

Proline concentration increased with the decrease in water content. Accumulation of amino acids and proline is a stress response from the perspective of altered photosynthetic metabolism. Accumulation of these solutes could react with the hydroxyl radicals thereby protecting lipids, DNA, proteins, and macro molecular structure from degradative reactions leading to cell destructions during drought (Orthen, *et al.*, 1994) [15]. Various experimental methods of water stress imposition can elicit proline accumulation in leaf tissue of young plants (Lawlor and Cornic, 2002) [17]. Proline acts as an osmoticum, a protective agent of enzyme and cellular structure and a storage compound of reducing nitrogen for rapid regrowth after stress are relieved (Misra, *et al.*, 2002) [23].

Material and Methods

Plant material source and design of experiment

Withania somnifera (L.) Dunal seeds were procured from the CIMAP, Hyderabad. Plants were grown in pot culture experiments with different treatments in black soil, a control without any addition to the soil, (Heavy metal treated) Treatment No. I Cadmium 10ppm, Chromium 20ppm, Nickel 16ppm were introduced into the soil, (Heavy metal + 1% (Ca OH)₂) Treatment No. II 1% of calcium hydroxide was also added along with heavy metals to soil. Then plants were grown up to the productivity levels in Green house of Botanical Garden, Department of Botany, Osmania University, Hyderabad, India.

Biochemical analysis

The plant materials (leaves) were collected from 90 day old plants. They leaves were washed with tap water followed by distilled water. The moisture on the plant material (leaves) surface was removed with the help of blotting paper. One gram of plant material (leaves) was thoroughly homogenized with 70% (v/v) ethyl alcohol. The homogenate was transferred to plastic bottles, labeled and stored in deep freezer for further biochemical analysis. However for chlorophyll estimation and enzyme assays fresh material was employed. The following metabolites were assayed in plants.

Estimation of soluble proteins

To 2.5 ml of ethanol homogenate, 2 ml of 10% (v/v) trichloroacetic acid was added and centrifuged at 4000 rpm for 10 minutes. The supernatant was discarded. The precipitate was dissolved in 5 ml of 1% sodium hydroxide and was centrifuged at 4000 rpm for 10 minutes. The supernatant was used for estimation of proteins by Lowry *et al.*, (1951) [19] method.

Estimation of carbohydrates

2.5 ml of ethanol homogenate was taken into centrifuge tubes. The tubes were kept in a boiling water bath for 5 minutes. After cooling, the contents were centrifuged at 4,000 rpm for 10 minutes. The supernatant was collected. The residue was re-extracted with 5 ml of 70% (v/v) ethanol and was centrifuged again. This procedure was repeated 3 times. The ethanol supernatants were pooled and made up to 10 ml. This was used for the estimation of total sugars and reducing sugars.

Estimation of total sugars

Total sugars were estimated according to the method (Yoshida *et al.*, 1976) [40]. 5 ml of alcohol extract was evaporated to dryness in a clean breaker in a water bath at 60⁰ C. The lipids and pigments were removed by washing the evaporated residue repeatedly with diethylether. Then the residue was dissolved in 5 ml of 40% (v/v) ethanol. This was used for the estimation of total sugars by anthrone reagent.

Estimation of reducing sugars

Reducing sugars were determined according to Nelson (1944) method. Nelson reagent was used for the estimation of reducing sugars.

Estimation of non-reducing sugars

The amount of non-reducing sugars was calculated by the following formulae as given by Loomis and Shull (1977).

$$\text{Non reducing sugars} = (\text{Total sugars} - \text{free reducing sugars}) \times 0.95$$

The amount of non- reducing sugars was expressed as glucose in terms of mg g⁻¹ fresh weight.

Estimation starch

Starch was estimated from the residue left after extraction of the sugar by employing the method of McCready, *et al.*, (1950).

Estimation of free proline

The plant samples (0.5gm) were homogenized with 10 ml of 3 % (w/v) aqueous sulfosalicylic acid and the homogenate was filtered through whatman No. 2 filter paper. The supernatant was taken for proline estimation. Proline content was estimated as described by Bates (1973) [3]. Ninhydrin reagent was used for the estimation of proline.

Estimation of phenols

Total phenols were extracted and estimated by using procedure of Swain and Hillis (1959).

Nucleic acids

DNA and RNA present in the ethanolic homogenate were separated by the procedure described (Ogur and Rosen, 1950) [14].

Estimation of RN

The RNA present in the extract was estimated according to the procedure of Schneider (1957) [31] with the use of orcinol reagent.

Estimation of DNA

The DNA present in the extract was estimated according to the procedure of Burton (1968) [4].

Enzymes

For the study of different enzymes, plant tissues like leaves were employed. Samples of plant tissue were primed from 30 days old plants grown in different plant tissue samples were washed with sterile distilled water, blotted dry and kept in the freezing compartment till they were used. Extractions of the enzymes were carried out in a cold room. The glassware which was used for extraction and assay of the enzymes were pre- chilled in a deep freezer. All the buffers and solutions used for the enzyme studies were prepared freshly and chilled before use.

Oxidizing enzymes (Extraction of catalase, peroxides and polyphenol oxidase)

200 mg of plant material was homogenized with 10 ml of 0.1 M phosphate buffer (pH = 7) in a pre-chilled mortar with pestle. The samples were centrifuged at 4°C for 15 minutes at 17,000 g in REMI T-8 centrifuge. The clear supernatant was used as the enzyme source for catalase, peroxidase and polyphenol oxidase.

Catalase

Catalase activity was assayed by adopting the method of Barder (1980).

Peroxidase

Peroxidase activity was assayed by employing the procedure of Kar and Mishra (1976) [2].

Polyphenol oxidase

Polyphenol oxidase activity was assayed according to the procedure of Kar and Mishra (1976) [2].

Result and Discussions

Analysis of various parameters in 40 days of old leaves i.e. after maturation (stable)

Soluble proteins content

The soluble protein content in the leaves of *Withania somnifera* (L.) Dunal was 2.504 ± 0.10 mg/gm when plants were grown in control soil, the plants grown in heavy metal treated soil was 1.77 ± 0.083 mg/gm., and in plants grown with heavy metal + 1% Ca(OH)₂ treated soil was 2.25 ± 0.12 mg/gm. The difference between the mean values of control plants and heavy metal treated plants differed significantly ($t = 0.36887$, $df = 10$, $P < 0.05$). The difference between the mean values of control plants and heavy metal + 1% Ca(OH)₂ treated plants were found significant ($t = 0.5846$, $df = 10$, $P < 0.05$). The difference between the mean values of heavy metal treated and heavy metal + 1% Ca(OH)₂ treated plants were found significant ($t = 0.2157$, $df = 10$, $P < 0.05$). The soluble protein content was reduced in heavy metal treated (Treatment No. I) plants when compared to control plants and heavy metal + 1% calcium hydroxide treated (Treatment No. II) plants.

Total sugars content

The total sugar content in the leaves of *Withania somnifera* (L.) Dunal was 1.92 ± 0.08 mg/gm when plants were grown in control soil, the plants grown in heavy metal treated soil was 2.023 ± 0.08 mg/gm, and in plants grown with heavy metal + 1% Ca(OH)₂ treated soil was 1.937 ± 0.04 mg/gm. The difference between the mean values of control plants and heavy metal treated plants differed significantly ($t = 1.630$, $df = 10$, $P < 0.05$). The difference between the mean values of control plants and heavy metal + 1% Ca(OH)₂ treated plants were found significant ($t = 1.390$, $df = 10$, $P < 0.05$). The difference between the mean values of heavy metal treated and heavy metal + 1% Ca(OH)₂ treated plants were found significant ($t = 0.2403$, $df = 10$, $P < 0.05$). The total sugar content was observed maximum in heavy metals treated (Treatment No. I) plants when compared to control plants and heavy metal + 1% calcium hydroxide treated (Treatment No. II) Plants.

Reducing sugars content

The reducing sugar content in the leaves of *Withania somnifera* (L.) Dunal was 1.615 ± 0.14 mg/gm when plants

were grown in control soil, the plants grown in heavy metal treated soil was 1.873 ± 0.13 mg/gm., and in plants grown with heavy metal + 1% Ca(OH)₂ treated soil was 1.696 ± 0.15 mg/gm. The difference between the mean values of control plants and heavy metal treated plants differed significantly ($t = 0.7815$, $df = 10$, $P < 0.05$). The difference between the mean values of control plants and heavy metal + 1% Ca(OH)₂ treated plants were found significant ($t = 0.5640$, $df = 10$, $P < 0.05$). The difference between the mean values of heavy metal treated and heavy metal + 1% Ca(OH)₂ treated plants were found significant ($t = 0.2174$, $df = 10$, $P < 0.05$). The reducing sugar content was increased in heavy metals treated (Treatment No. I) plants when compared to control plants and heavy metal + 1% calcium hydroxide treated (Treatment No. II) Plants.

Non-reducing sugars content

The non-reducing sugar content in the leaves of *Withania somnifera* (L.) Dunal was 0.281 ± 0.02 mg/gm when plants were grown in control soil, the plants grown in heavy metal treated soil was 0.140 ± 0.01 mg/gm, and in plants grown with heavy metal + 1% Ca(OH)₂ treated soil was 0.221 ± 0.01 mg/gm. The difference between the mean values of control plants and heavy metal treated plants differed significantly ($t = 2.107$, $df = 10$, $P < 0.05$). The difference between the mean values of control plants and heavy metal + 1% Ca(OH)₂ treated plants were found significant ($t = 1.3549$, $df = 10$, $P < 0.05$). The difference between the mean values of heavy metal treated and heavy metal + 1% Ca(OH)₂ treated plants were found significant ($t = 0.7526$, $df = 10$, $P < 0.05$). The non-reducing sugar content was observed as minimum in heavy metals treated (Treatment No. I) plants when compared to control plants and heavy metal + 1% calcium hydroxide treated (Treatment No. II) Plants.

DNA content

The DNA content in the leaves of *Withania somnifera* (L.) Dunal was 5.153 ± 0.50 mg/gm when plants were grown in control soil, the plants grown in heavy metal treated soil was 4.563 ± 0.11 mg/gm, and in plants grown with heavy metal + 1% Ca(OH)₂ treated soil was 5.038 ± 0.50 mg/gm. The difference between the mean values of control plants and heavy metal treated plants differed significantly ($t = 0.9356$, $df = 10$, $P < 0.05$). The difference between the mean values of control plants and heavy metal + 1% Ca(OH)₂ treated plants were found significant ($t = 0.0560$, $df = 10$, $P < 0.05$). The difference between the mean values of heavy metal treated and heavy metal treated + 1% Ca(OH)₂ barrier plants were found significant ($t = 0.8796$, $df = 10$, $P < 0.05$). The DNA content was maximum in control plants when compared to heavy metals treated (Treatment No. I) plants and heavy metal + 1% calcium hydroxide treated (Treatment No. II) Plants.

RNA content

The RNA content in the leaves of *Withania somnifera* (L.) Dunal was 4.88 ± 0.32 mg/gm. when plants were grown in control soil, the plants grown in heavy metal treated soil was 2.711 ± 0.28 mg/gm., and in plants grown with heavy metal + 1% Ca(OH)₂ treated soil was 4.855 ± 0.48 mg/gm. The difference between the mean values of control plants and heavy metal treated plants differed significantly ($t = 0.20096$, $df = 10$, $P < 0.05$). The difference between the mean values of control plants and heavy metal + 1% Ca(OH)₂ treated plants were found significant ($t = 0.0258$, $df = 10$, $P < 0.05$). The difference between the mean values of heavy metal treated

and heavy metal treated + 1% Ca(OH)₂ barrier plants were found significant ($t = 0.1757$, $df = 10$, $P < 0.05$). The RNA content was minimum in heavy metals treated (Treatment No. I) plants when compared to control plants and heavy metal + 1% calcium hydroxide treated (Treatment No. II) plants.

Phenols content

The phenol contents in the leaves of *Withania somnifera* (L.) Dunel was $4.379 \pm 0.0\text{mg/gm}$. when plants were grown in control soil, the plants grown in heavy metal treated soil was $3.638 \pm 0.13\text{mg/gm}$, and in plants grown with heavy metal + 1% Ca(OH)₂ treated soil was $3.992 \pm 0.85\text{mg/gm}$. The difference between the mean values of control plants and heavy metal treated plants differed significantly ($t = 0.7536$, $df = 10$, $P < 0.05$). The difference between the mean values of control plants and heavy metal + 1% Ca(OH)₂ treated plants were found significant ($t = 1.1425$, $df = 10$, $P < 0.05$). The difference between the mean values of heavy metal treated and heavy metal treated + 1% Ca(OH)₂ barrier plants were found significant ($t = 0.3889$, $df = 10$, $P < 0.05$). The phenol content was reduced in heavy metals treated (Treatment No. I) plants when compared to control plants and heavy metal + 1% calcium hydroxide treated (Treatment No. II) Plants.

Free proline content

The free proline content in the leaves of *Withania somnifera* (L.) Dunel was $21.46 \pm 1.66\text{mg/gm}$. when plants were grown in control soil, the plants grown in heavy metal treated soil was $16.543 \pm 2.90\text{mg/gm}$, and in plants grown with heavy metal + 1% Ca(OH)₂ treated soil was $25.27 \pm 3.32\text{mg/gm}$. The difference between the mean values of control plants and heavy metal treated plants differed significantly ($t = 0.334$, $df = 10$, $P < 0.05$). The difference between the mean values of control plants and heavy metal + 1% Ca(OH)₂ treated plants were found significant ($t = 0.1095$, $df = 10$, $P < 0.05$). The difference between the mean values of heavy metal treated and heavy metal treated + 1% Ca(OH)₂ barrier plants were found significant ($t = 0.4429$, $df = 10$, $P < 0.05$). The free proline content was maximum in heavy metal + 1% calcium hydroxide treated (Treatment No. II) Plants compared to heavy metals treated (Treatment No. I) plants and control plants.

Starch content

The starch content in the leaves of *Withania somnifera* (L.) Dunel was $0.79 \pm 0.02\text{mg/gm}$. when plants were grown in control soil, the plants grown in heavy metal treated soil was $0.73 \pm 0.054\text{mg/gm}$, and in plants grown with heavy metal + 1% Ca(OH)₂ treated soil was $0.75 \pm 0.051\text{mg/gm}$. The difference between the mean values of control plants and heavy metal treated plants differed significantly ($t = 1.676$, $df = 10$, $P < 0.05$). The difference between the mean values of control plants and heavy metal + 1% Ca(OH)₂ treated plants were found significant ($t = 1.3782$, $df = 10$, $P < 0.05$). The difference between the mean values of heavy metal treated and heavy metal treated + 1% Ca(OH)₂ barrier plants were found significant ($t = 1.41828$, $df = 10$, $P < 0.05$). The starch content was minimum in heavy metal treated (Treatment No. I) plants when compared to other treatments.

Catalase activity

The catalase activity in the leaves of *Withania somnifera* (L.) Dunel was $1.00 \pm 0.15\text{mg/gm}$. when plants were grown in control soil, the plants grown in heavy metal treated soil was

$0.835 \pm 0.1\text{mg/gm}$, and in plants grown with heavy metal + 1% Ca(OH)₂ treated soil was $0.961 \pm 0.1\text{mg/gm}$. The difference between the mean values of control plants and heavy metal treated plants differed significantly ($t = 0.4587$, $df = 10$, $P < 0.05$). The difference between the mean values of control plants and heavy metal + 1% Ca(OH)₂ treated plants were found significant ($t = 0.06574$, $df = 10$, $P < 0.05$). The difference between the mean values of heavy metal treated and heavy metal treated + 1% Ca(OH)₂ barrier plants were found significant ($t = 0.5245$, $df = 10$, $P < 0.05$). The catalase activity was decreased in heavy metals treated (Treatment No. II) plants when compared to heavy metal + 1% calcium hydroxide treated (Treatment No. II) Plants and control plants.

Peroxidase activity

The peroxidase activity in the leaves of *Withania somnifera* (L.) Dunel was $0.0103 \pm 0.001\text{ mg/gm}$. when plants were grown in control soil, the plants grown in heavy metal treated soil was $0.0078 \pm 0.001\text{ mg/gm}$, and in plants grown with heavy metal + 1% Ca(OH)₂ treated soil was $0.0109 \pm 0.001\text{mg/gm}$. The difference between the mean values of control plants and heavy metal treated plants differed significantly ($t = 0.9388$, $df = 10$, $P < 0.05$). The difference between the mean values of control plants and heavy metal + 1% Ca(OH)₂ treated plants were found significant ($t = 0.2517$, $df = 10$, $P < 0.05$). The difference between the mean values of heavy metal treated and heavy metal treated + 1% Ca(OH)₂ barrier plants were found significant ($t = 0.6871$, $df = 10$, $P < 0.05$). The peroxidase activity was decreased in heavy metals treated (Treatment No. I) plants when compared to heavy metal + calcium hydroxide treated (Treatment No. II) plants and control plants.

Polyphenol oxidase activity

The polyphenol oxidase activity in the leaves of *Withania somnifera* (L.) Dunel was $0.313 \pm 0.009\text{ mg/gm}$ when plants were grown in control soil, the plants grown in heavy metal treated soil was $0.481 \pm 0.02\text{ mg/gm}$, and in plants grown with heavy metal + 1% Ca(OH)₂ treated soil was $0.36 \pm 0.03\text{mg/gm}$. The difference between the mean values of control plants and heavy metal treated plants differed significantly ($t = 0.30102$, $df = 10$, $P < 0.05$). The difference between the mean values of control plants and heavy metal treated + 1% Ca(OH)₂ barrier plants were found significant ($t = 0.1781$, $df = 10$, $P < 0.05$). The difference between the mean values of heavy metal treated and heavy metal + 1% Ca(OH)₂ treated plants were found significant ($t = 0.1228$, $df = 10$, $P < 0.05$). The peroxidase activity was increased in heavy metals treated (Treatment No. I) plants when compared to heavy metal + calcium hydroxide treated (Treatment No. II) Plants and control plants.

The heavy metal treated plants (Treatment No. I) showed lowest in soluble protein contents, non-reducing sugar contents, RNA contents, DNA contents, phenol content, free proline contents, catalase and peroxides activity and also supported maximum in total sugar contents, reducing sugar contents and polyphenol oxidase activity as compared to control plants and heavy metal + 1% calcium hydroxide treated (Treatment No. II) plants. In plants that due to heavy metals (Cd, Cu, Cr, Ni, Pb, Hg, Pt and Zn) stress, there is reduced efficiency of DNA synthesis, weaker DNA protection from damaged chromatin protein (histone) and increased deoxyribonuclease (DNase) activity (Prasad and Strzalka, 2002) [25]. The metals such as Cu, Ni, Cd and Pb have been

reported to decrease RNA synthesis and to active ribonuclease in RNA content (Schmidt, 1996). The sugar and starch content showed a decreasing trend with progressive increase in zinc content in cluster Bean (Manivasagaperumal, *et al.*, 2011) [20] and carbohydrate catabolism (Rabie *et al.* 1992) [26]. The content of free phenol was found to increase in wheat with increasing Cu^{2+} concentration in the medium (Ganeva and Zozikova 2007) [11]. Enhanced protein hydrolysis resulting in decreased concentration of soluble proteins (Menlnichuk, *et al.*, 1982). Proline has increased with stress of heavy metals (Zengin and Kirbag 2007) [41]. Proline accumulation under stress condition may either caused by induction or activation

of enzymes of proline biosynthesis or a decreased proline oxidation to glutamate, decreased utilization of proline in protein synthesis, and enhanced protein turnover (Delauney and Verna, 1993) [9]. The phenol content also a sewage against hazardous oxidative damage to plant cells (Karadeniz, *et al.*, 2005) and antioxidant activity (Cao, *et al.*, 1996; Ness and Powless 1997) [8, 24]. These finding are in agreement with earlier reports (Jothimani, *et al.*, 2002; Ramachandran, 1994; Saravanamoorthy and Ranjith Kumar, 2002; Swaminathan and Vaidheeswarwan, 1991; Veer and Lats, 1987) [13, 27, 29, 37, 38].

Table 1: Biochemical analysis and enzyme activity expressed as leaves of *Withania somnifera* (L.). Dunel plants grown in three treatments (mg/gm. fresh weight)

Parameters	Control	Treatment No. I (Soil + Heavy metal treated)	Treatment No. II (Soil + Heavy metal + 1% $\text{Ca}(\text{OH})_2$)
	Mean \pm SD	Mean \pm SD	Mean \pm SD
Soluble proteins	2.504 \pm 0.10	1.77 \pm 0.083	2.25 \pm 0.12
Total sugars	1.92 \pm 0.08	2.023 \pm 0.08	1.937 \pm 0.04
Reducing sugars	1.615 \pm 0.14	1.873 \pm 0.13	1.696 \pm 0.15
Non reducing sugars	0.281 \pm 0.02	0.140 \pm 0.01	0.221 \pm 0.01
DNA content	5.153 \pm 0.50	4.563 \pm 0.11	5.038 \pm 0.50
RNA content	4.88 \pm 0.32	2.711 \pm 0.28	4.855 \pm 0.48
Phenols	4.379 \pm 0.09	3.638 \pm 0.13	3.992 \pm 0.15
Free Proline	21.46 \pm 1.66	16.543 \pm 2.90	25.27 \pm 3.32
Starch	0.79 \pm 0.026	0.73 \pm 0.051	0.75 \pm 0.054
Catalase	1.00 \pm 0.15	0.835 \pm 0.1	0.961 \pm 0.1
Peroxidase	0.0103 \pm 0.001	0.0078 \pm 0.001	0.0109 \pm 0.001
Polyphenol oxidase	0.313 \pm 0.009	0.481 \pm 0.02	0.36 \pm 0.03

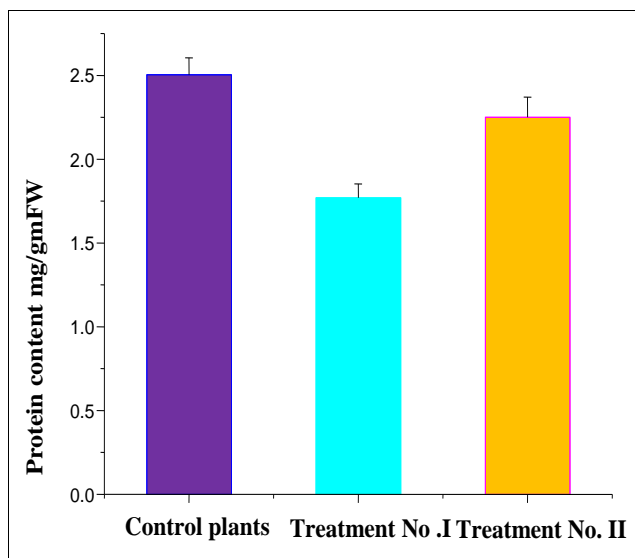


Fig 1: Protein content (mg/gm. of fresh weight) in the leaves of *Withania somnifera* (L.). Dunel plants were grown in three treatments

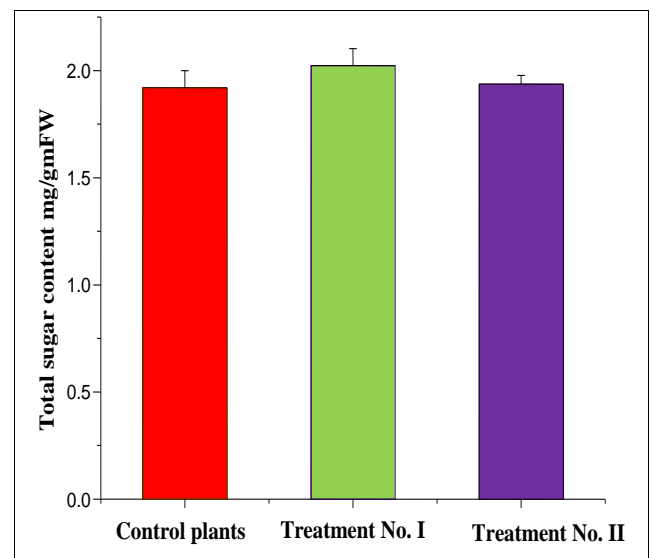


Fig 2: Total sugar content (mg/gm. of fresh weight) in the leaves of *Withania somnifera* (L.). Dunel plants were grown in three treatments

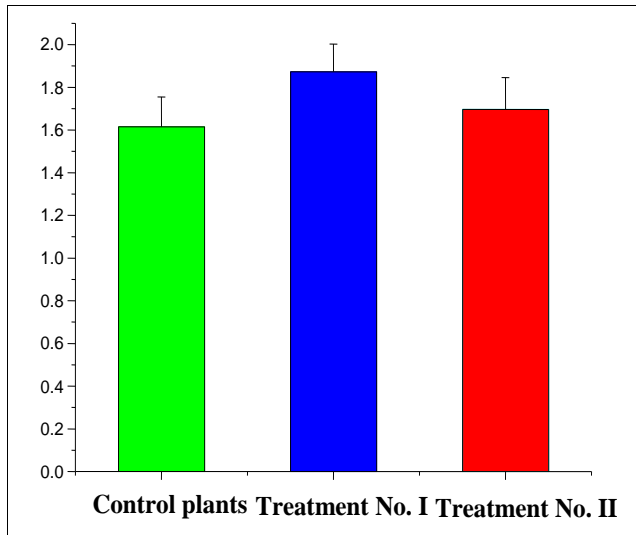


Fig 3: Reducing sugar content (mg/gm. of fresh weight) in the leaves of *Withania somnifera* (L.). Dunel plants were grown in three treatments

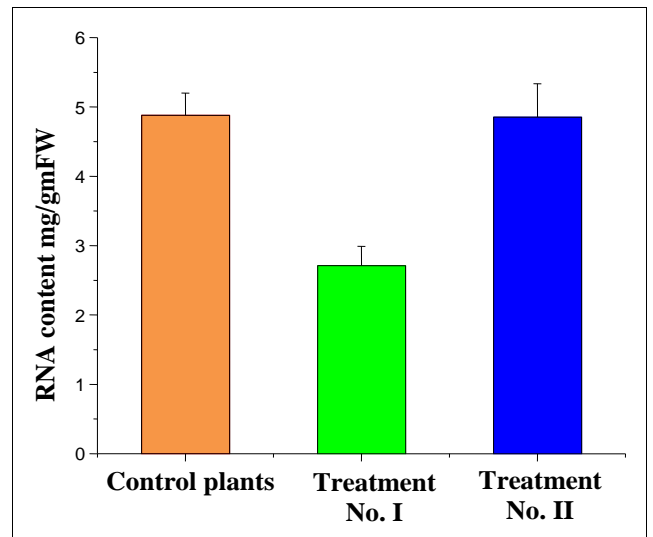


Fig 6: RNA content (mg/gm. of fresh weight) in the leaves of *Withania somnifera* (L.). Dunel plants were grown in three treatments

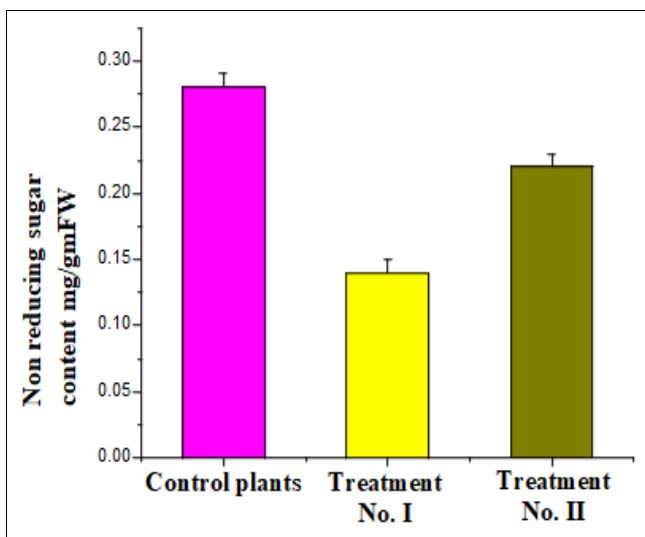


Fig 4: Non reducing sugar content (mg/gm. of fresh weight) in the leaves of *Withania somnifera* (L.). Dunel plants were grown in three treatments

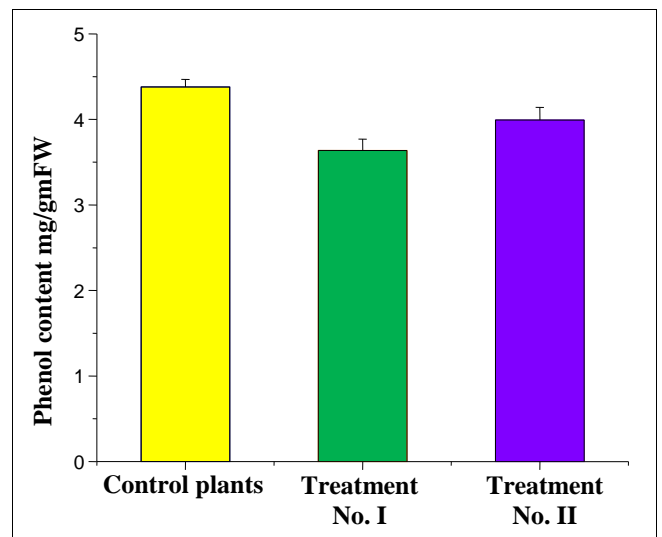


Fig 7: Phenol content (mg/gm. of fresh weight) in the leaves of *Withania somnifera* (L.) Dunel. plants were grown in three treatments

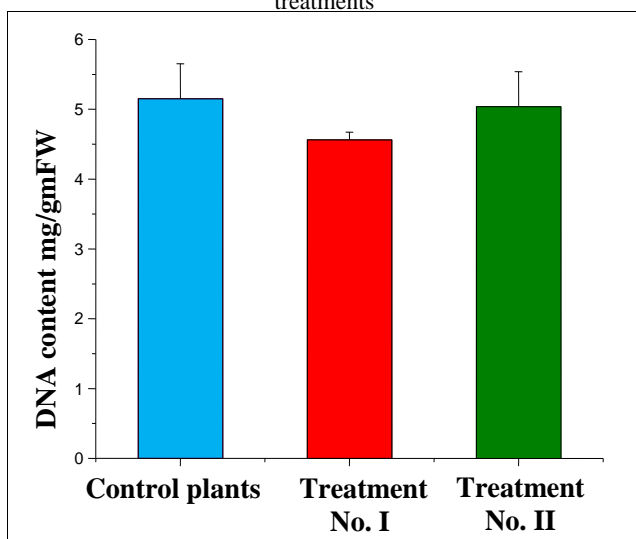


Fig 5: DNA content (mg/gm. of fresh weight) in the leaves of *Withania somnifera* (L.). Dunel plants were grown in three treatments

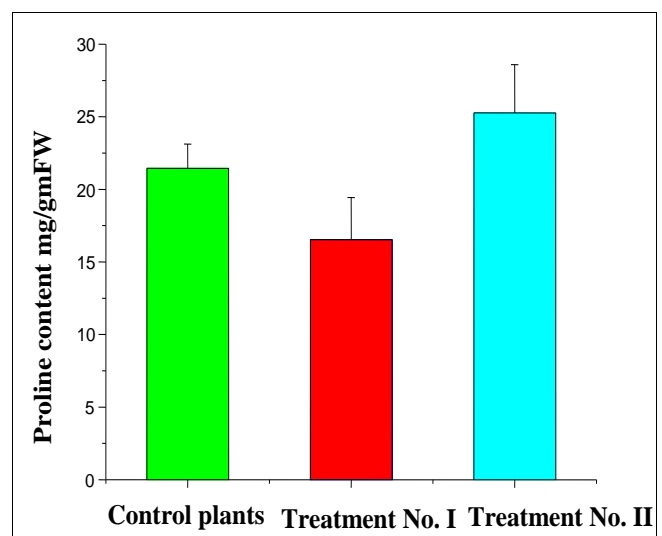


Fig 8: Proline content (mg/gm. of fresh weight) in the leaves of *Withania somnifera* (L.). Dunel plants were grown in three treatments

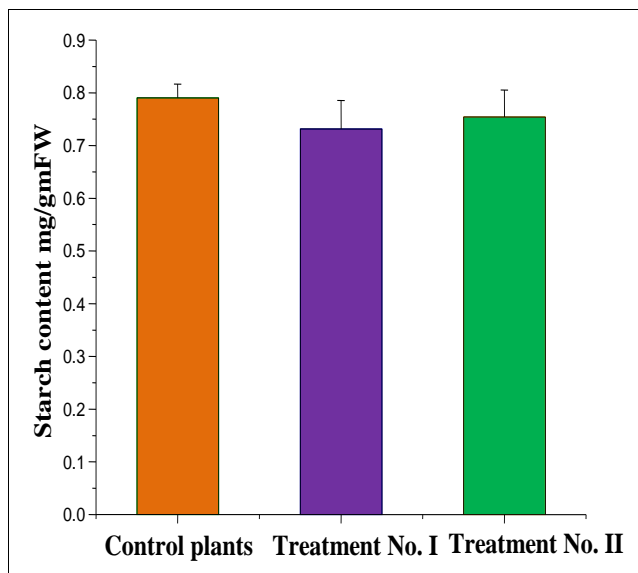


Fig 9: Starch content (mg/gm. of fresh weight) in the leaves of *Withania somnifera* (L.). Dunel plants were grown in three treatments

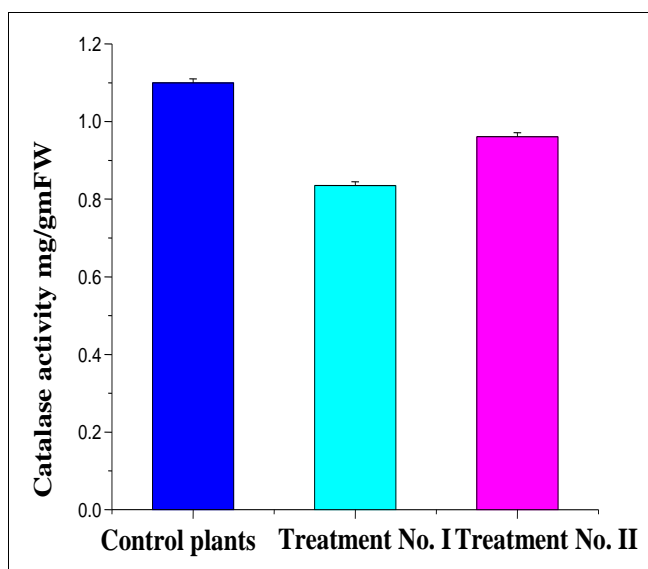


Fig 10: Catalase activity (mg/gm. of fresh weight) in the leaves of *Withania somnifera* (L.). Dunel plants were grown in three treatments

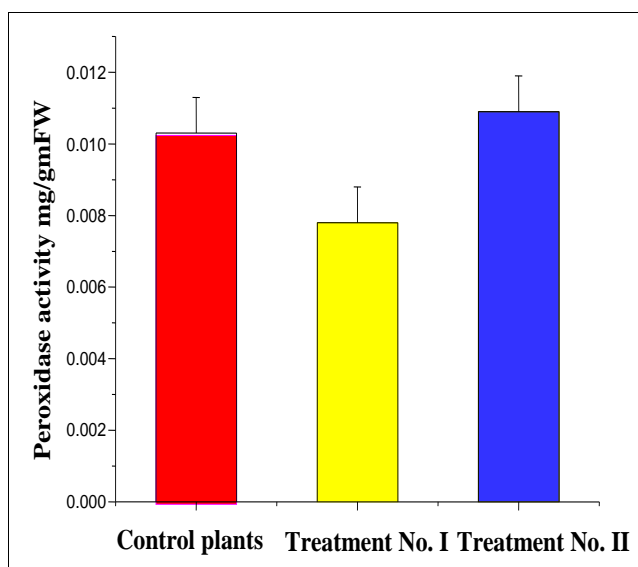


Fig 11: Peroxidase activity (mg/gm. of fresh weight) in the leaves of *Withania somnifera* (L.). Dunel plants were grown in three treatments

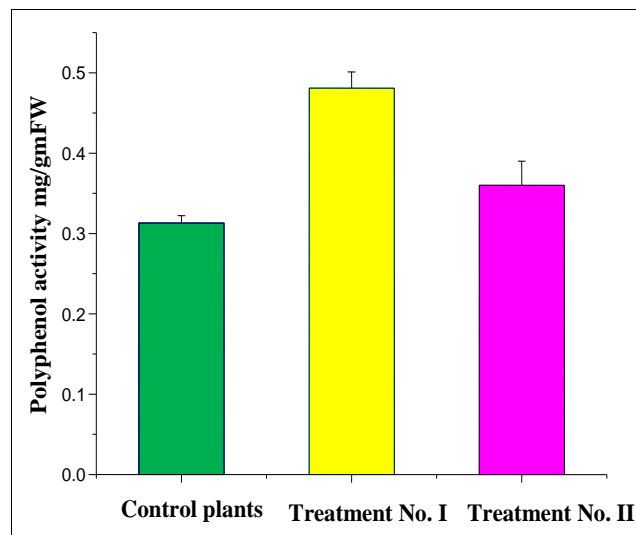


Fig 12: Polyphenol activity (mg/gm. of fresh weight) in the leaves of *Withania somnifera* (L.). Dunel plants were grown in three treatments

Conclusion

The heavy metal treated plants (Treatment No. I) showed lowest in soluble protein contents, non-reducing sugar contents, RNA contents, DNA contents, phenol content, free proline contents, catalase and peroxides activity when compared to other treatments. The heavy metal treated plants (Treatment No. I) supported maximum in total sugar contents, reducing sugar contents and polyphenol oxidase activity when compared to control plants and heavy metal + 1% calcium hydroxide treated (Treatment No. II) Plants.

The results suggest that medicinal plants used for human consumption or for preparation of herbal products and standardized extracts should be collected from an unpolluted natural habitat.

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