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Effect of Heavy Metals (Cu, Zn and Cr) on Total Carbohydrate and Protein Content in *Andrographis paniculata* (Burm. f.) Wall. ex Nees

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Abstract
Andrographis paniculata *(Burm. f.) Wall. ex Nees is a medicinal plant that is used to treat various diseases like diabetes, hepatitis, and fever. The current study is aimed to identify the effect of three selected heavy metals Copper, Zink, and Chromium on the total carbohydrate and protein content in Andrographis paniculata. Concentrations selected for the analysis are 25ppm, 50ppm, 75ppm, 100ppm, 125ppm, 150ppm and 175ppm. Heavy metal treatment was done in the hydroponics method which was carried out in glass bottles. Modified Hogland Solution (Epstein, 1972) was used as a nutrient medium for hydroponics culture. Plant samples for the carbohydrate and protein content estimation were collected at 10-day intervals. The study shows that 25 ppm Cu causes a very slight increase in carbohydrate content as compared to control and from 50 ppm Cu treatment causes a lowering of carbohydrate content. Zn and Cr cause a lowering of carbohydrate content even from 25ppm. The protein content is found to be increased in all three heavy metal treated plants at lower concentrations. From the concentration higher than 125 ppm Cu and Zn cause lowering of protein content. The plants treated with Cr show a lowering of protein content from 75ppm concentration.*

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1. INTRODUCTION

After the industrial revolution pollution of the biosphere by toxic chemicals and heavy metals was intensified rigorously. The increased amount of heavy metals can cause various physiological and biochemical modifications which can negatively impact ecosystem stability. Combustion of fissile fuels, mining, smelting of metalliferous ores, municipal waste disposal, and agricultural practices like the usage of pesticides and weedicides are the major source of heavy metal pollution. Heavy metal pollution is hazardous for both fauna and flora. Several heavy metals at their lower concentrations act as micronutrients for plants but when the concentration exceeds optimal level they negatively affect plats growth and metabolism. Copper is essential for the growth and development of plants, it is a catalyst for fundamental processes like photosynthesis and respiration but at higher concentrations, copper is extremely toxic to plants and causes symptoms like chlorosis, necrosis, and inhibition of growth (Bradl, 2005). Zink is one of the major micronutrients for plants which is involved in many physiological processes like nitrogen metabolism, photosynthesis, and

resistance against abiotic and biotic stress. Chromium is widely used for different industrial purposes like electroplating. Alloying, leather tanning, and manufacturing of paints so the chance of chromium reaching into the ecosystem is very high. Chromium exists in different oxidation states but Cr(0), Cr(6), and Cr(3) are the most stable oxidation states. Chromium is toxic to plants even at very low concentrations. *Andrographis paniculata* (Acanthaceae) is a medicinal plant that is widely used in Ayurvedic and Chinese medicine for the treatment of liver disorders, colic pain in children fever, bug bite, dysentery, and malaria. It is an annual herb with an extremely bitter taste. Studies in *Andrographis paniculata* show a broad range of pharmacological properties including antidiarrheal (Gupta, Choudhry, Yadava, Srivastava & Tandon, 1990), anti-hepatitis (Sharma et al, 1991), antioxidant (Kamderm et al, 2002), antimicrobial (Wiart et al, 2005) immunostimulatory (Calabrese et al., 2000), and anti-inflammatory (Chiou, Lin & Chen, 1998).

2. MATERIALS AND METHOD

Andrographis paniculata seeds were collected from healthy-looking plants from the Botanical garden, Sree Narayana College, Nattika, Thrissur, Kerala, India. Seeds were germinated in the potting mixture. 14 days after the germination healthy seedlings with uniform morphological features were selected for heavy metal treatment and transferred to hydroponics systems prepared using good quality glass butties of size 8.5 Cm height and 5.5 Cm Diameter. Modified Hogland Solution (Epstein, 1972) was used for hydroponics culture of *Andrographis paniculata.* Stock solutions of each nutrient was prepared and appropriate volume was mixed as mentioned by Taiz and Zeiger (2002) PH of the solution was adjusted to 6.8 using 0.1 N HCL. 25ppm, 50ppm, 75ppm, 100ppm, 125ppm, 150ppm, and 175ppm concentrations of Copper, Zink, and Chromium were prepared from $CuSO_4$, $ZnSO_4$, and $Cr_2(SO_4)_3$ using Modified Hogland Solution as solvent and from that 100 ml from each heavy metal solutions were transferred to labeled glass jars. The hydroponic system was maintained indoors under Full Spectrum 220V LED Grow Light. Bottles containing only nutrient solutions were taken as control. A minimum of 10 bottles were kept for all treatments to get sufficient plant samples for analysis. Samples of each treatment were collected on the 10th 20th and 30th days of growth. At each intervals, harvested plants were washed thoroughly in distilled water and blotted dry. After drying in shade the whole plant homogenized by powdering using mortar and pestle. Total carbohydrate content present in *Andrographis paniculata* after treatment of three heavy metals at various concentrations was estimated using the phenol sulphuric acid method of carbohydrate estimation reported by (Masuko et al, 2005). Protein content in *Andrographis paniculata* after heavy metals treatment was estimated according to (Lowry et al, 1951) using Bovin Serum Albumin (66 KDa) as standard.

3. RESULTS

Table 1: Effect of heavy metals on carbohydrate content in *Andrographis paniculata* (mg /g dry weight)

	Interval	C	25ppm	50ppm	75ppm	100ppm	125ppm	150ppm	175ppm
Cu	10	42.77±.09	44.27±.11	41.28±.04	37.29±.15	35.37±.12	30.47±.05	27.35±.13	25.33±.09
	20	51.8±.11	53.73±.13	50.27±	40.37±.06	37.3±.09	31.47±.12	30.11±.11	27.08±.07
	30	64.7±.14	65.27±.09	59.28±12	48.37±.08	40.36±.14	33.47±.16	31.37±.07	28.96±.15
Zn	10	42.77±.09	43.18±.07	41.02±.14	36.27±.13	31.93±.11	28.31±.06	26.32±.16	24.49±.11
	20	51.8±.11	48.29±.15	44.27±.15	38.2±.07	33.27±.07	30.17±.11	28.39±.07	26.49±.15
	30	64.7±.14	59.28±.11	47.28±.13	40.28±.12	35.63±.14	32.47±.14	31.39±.09	29.56±.09
Cr	10	42.77±.09	40.28±.06	39.47±.06	36.49±.08	30.29±.05	27.39±.08	25.48±.14	22.48±.11
	20	51.8±.11	48.29±.08	47.29±.15	38.49±.15	34.5±.16	30.48±.12	28.38±.07	24.49±.14
	30	64.7±.14	56.34±.04	54.68±.13	39.48±.14	36.04±.14	31.97±.11	30.75±.07	25.07±.10

Values are mean of 5 replicates ± standard error

Table 2: Effect of heavy metals on protein content in in *Andrographis paniculata* (mg / g dry weight)

	Interval	C	25ppm	50ppm	75ppm	100ppm	125ppm	150ppm	175ppm
Cu	10	12.37±.03	12.84±.12	13.48 ±.07	14.85±.09	12.48±..15	10.38±.13	8.349±.,14	6.48±.15
	20	14.56±.02	14.83±.09	15.38±.13	15.82±.13	12.9±.068	11.38±.11	8.39±07	7.39±.09
	30	17.23±.06	20.32±.12	17.39±.06	16.32±.16	14.5±.118	12.49±,07	10.04±.11	8.4±.07
Zn	10	12.37±.03	13.34±.17	14.65±.09	16.13±.08	12.3±.072	10.32±.16	8.74±.06	6.23±.16
	20	14.56±.02	16.17±.07	18.39±.11	19.29±.07	14.2±.119	12.84±.07	9.2±.13	7.38±.17
	30	17.23±.06	21.38±.11	23.44±.13	20.29±.14	15.3±.089	13.49±.09	11.46±.07	8.49±.09
Cr	10	12.37±.03	13.94±.14	15.38±.07	12.07±.14	11.4±.147	9.39±.11	7.34±.16	5.28±.11
	20	14.56±.02	16.49±.08	17.82±.08	14.18±.16	13.4±.08	11.37±.13	8.52±.11	6.25±.15
	30	17.23±.06	22.49±.15	18.25±.14	15.68±.09	14.59±.16	13.48±.14	9.02±.15	6.92±.08

Values are mean of 5 replicates ± standard error

4. DISCUSSION

Carbohydrate is an important molecule that is necessary for plants. It is the reserved energy for the growth and development of plants. The presence of heavy metals like Copper, Zinc, and Chromium is found to affect the carbohydrate content in *Andrographis paniculata.* The total carbohydrate content on the 10th-day interval is 42.77mg in control. Carbohydrate content in plant samples treated with Cu and Zn is found to be slightly increased as compared to control but Chromium treatment causes a slight reduction in carbohydrate content even at the lowest concentration. As the period of treatment increases, Zink and Chromium show a decrease in carbohydrate content when compared with control. At 25ppm Copper, treatments induce a slight increase in carbohydrate content at all three intervals. At Concentrations higher than 25 ppm all three heavy metals cause reduction in carbohydrate content. At the highest concentration chromium is found to have a maximum adverse effect on carbohydrate content which is followed by Zink and the least effect is found to be in plants treated with Copper

In comparison with Control total protein content in *Andrographis paniculata* shows a slight increase in plants that were treated with a lower concentration of Cu, Zn, and Cr. As the concentration of Heavy metals increase considerable reduction in protein content was found. In plants treated with 25ppm heavy metal concentration, Cr treated samples show more increased protein content which is followed by Zink and then Copper. Plants treated with Cr show a decrease in protein content from 75 ppm concentration but in plants treated with Cu and Zn protein content was reduced from 125ppm and above concentrations. At concentrations above 125 ppm, all three heavy metals cause a noticeable reduction in the Total protein content of *Andrographis paniculata.* At highest concentration Cr cause, a maximum decrease in protein content, and plants treated with Cu shows higher protein concentration compared with Cr and Zn.

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6. CONCLUSION

Andrographis paniculata grown in presence of Cu, Zn and Cr show changes in total carbohydrate and protein content as compared to control. In the case of all three heavy metal treatments, Carbohydrate content was found to be decreasing as the concentration and the period of exposure increased. Cu, Zn, and Cr cause a slight increase in protein content at their lower concentrations as the concentration and period of treatment increases protein content is found to be decreasing

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