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PHYSIOLOGICAL AND BIOCHEMICAL ALTERATIONS IN *ANDROGRAPHIS PANICULATA* UNDER CADMIUM AND SODIUM STRESS

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Abstract-

Abiotic stresses such as heavy metal contamination and salinity severely affect the growth, physiology, and metabolic homeostasis of medicinal plants. The present study investigates the physiological and biochemical alterations in *Andrographis paniculata* under cadmium (Cd) and sodium (NaCl) stress**.** Uniform seedlings were subjected to varying concentrations of $CdCl_2$ and NaCl under controlled growth conditions. Physiological parameters including plant height, biomass accumulation, chlorophyll content, and relative water content were significantly reduced with increasing stress intensity. Both Cd and sodium stress induced excessive generation of reactive oxygen species, as evidenced by elevated levels of hydrogen peroxide and malondialdehyde, indicating enhanced lipid peroxidation. In response, antioxidant defence systems were markedly activated, with increased activities of superoxide dismutase, catalase, and peroxidase, although prolonged or higher stress levels caused partial enzyme inhibition. Biochemical analysis revealed a decline in total soluble protein and carbohydrate content, while stress-induced modulation of secondary metabolites, particularly andrographolide and total phenolics, was observed. Cadmium stress exerted more pronounced toxic effects compared to sodium stress, while combined responses highlighted the adaptive potential of *A. paniculata* through metabolic reprogramming and antioxidant regulation. The findings provide valuable insights into stress tolerance mechanisms in *A. paniculata* and have implications for its cultivation in metal- and salt-affected soils.

Keywords- Cadmium, Heavy metal, Physiological, Biochemical, *A. paniculata*, Stress.

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Introduction- *Andrographis paniculata* is important medicinal plants that have been widely recognized for their therapeutic potential and have been utilized in various traditional medicinal systems such as Ayurveda, Siddha, Unani, and Traditional Chinese Medicine since ancient times. Both species have a long history of use in treating a broad range of ailments, reflecting their pharmacological richness and bioactive potential. *A. paniculata* (commonly known as "Kalmegh" or "King of Bitters") belongs to the family Acanthaceae and is renowned for its bitter diterpenoid compounds, particularly andrographolide, which is responsible for many of its medicinal properties[1,2].

Plants are constantly exposed to a variety of abiotic stresses in their natural and agricultural environments, including extreme temperatures, excessive light intensity, drought, soil salinity, and contamination by heavy metals. Each of these stress factors interferes with normal physiological functions and ultimately restricts plant growth, development, and productivity. Among these stresses, salinity is one of the most widespread problems, especially in arid and semi-arid regions. High salt concentrations—primarily from sodium chloride (NaCl)—limit the plant's ability to absorb water, disturb osmotic equilibrium, and cause significant ionic imbalance. As Na^+ and Cl^- ions accumulate excessively in plant tissues, the uptake of essential minerals such as K^+, Ca^{2+}, and Mg^{2+} is reduced, disrupting enzymatic activities, membrane stability, and metabolic pathways[3,4].

The ionic toxicity and osmotic stress caused by salinity lead to excessive generation of reactive oxygen species (ROS), which in turn damage lipids, proteins, and nucleic acids, resulting in oxidative stress. To counter these harmful effects, plants activate a range of defence mechanisms. These include the upregulation of antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase, as well as the accumulation of Osmo protective compounds like proline, soluble sugars, glycine betaine, and other compatible solutes. These molecules help maintain cellular osmotic balance and protect biomolecules from stress-induced damage. However, allocating energy and resources to stress tolerance often comes at the cost of reduced growth, biomass production, and overall yield[5,6,7].

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Cadmium (Cd) is a pervasive environmental pollutant with no known plant metabolic function. Even at low concentrations, Cd can perturb nutrient uptake, disrupt photosynthetic processes, and generate oxidative stress via excess ROS production. Cd toxicity commonly results in reduced growth, chlorosis, impaired photosynthesis, and altered secondary metabolism in plants. Similarly, heavy metal contamination—particularly cadmium (Cd) originating from industrial effluents, mining activities, and polluted irrigation water—poses a severe threat to plant health. Cadmium is highly mobile in soil and can be readily absorbed by plant roots, where it interferes with nutrient uptake, disrupts chloroplast structure, inhibits photosynthetic pigments, and suppresses essential metabolic enzymes. As a result, plants exposed to cadmium commonly exhibit stunted growth, root shortening, leaf chlorosis, reduced photosynthetic efficiency, and impaired physiological performance. Both salinity and heavy metal stress ultimately weaken plant Vigor, compromise productivity, and can influence the quality and safety of plant-derived products, particularly in medicinal and edible species[8,9].

Phytochemical and pharmacological investigations have demonstrated that these plants possess a wide spectrum of biological activities, including anti-cancer, anti-inflammatory, anti-angiogenic, anti-malarial, anti-bacterial, antioxidant, hepatoprotective, and anti-hyperglycaemic properties. The presence of diverse secondary metabolites such as flavonoids, alkaloids, saponins, phenolics, glycosides, and terpenoids contributes to their medicinal value and therapeutic versatility. *A. paniculata* has been extensively studied for its potential in modulating immune responses and combating oxidative stress. These pharmacological activities not only validate their traditional use but also highlight their potential for modern drug development and phytopharmaceutical applications[10,11].

In recent decades, environmental contamination has emerged as a major global concern, and the accumulation of heavy metals in soil is now recognized as one of the most critical threats to the quality, safety, and therapeutic efficacy of medicinal plants. Rapid industrialization, expanding urban settlements, mining activities, and the widespread use of untreated or partially treated wastewater for irrigation have significantly accelerated the deposition of toxic metals in agricultural ecosystems. As a result, elements such as cadmium (Cd), lead (Pb), arsenic (As), and mercury

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(Hg) frequently accumulate in the top layers of soil where medicinal plants are cultivated.

Table 1- Effect of some toxic metals for different type of plants (Al-Khayri *et al.*, 2023)

Metal	Typical contaminated medium	Effect on vegetative growth	Effect on reproductive growth	Example plants reported affected
Lead (Pb)	Contaminated soil (industrial sites, leaded petrol residue)	Reduced root and shoot growth, chlorosis, impaired nutrient uptake, reduced photosynthesis	Reduced flowering, lower pollen viability and seed set	*Zea mays, Brassica juncea, Phaseolus spp.*
Arsenic (As)	Groundwater/soil (mining, pesticide residues)	Root growth inhibition, leaf chlorosis and necrosis, reduced biomass, impaired water relations	Reduced flower production, abnormal seeds, lower germination in offspring	*Oryza sativa (rice), Pteris vittata (fern hyperaccumulator)*
Mercury (Hg)	Soil & atmospheric deposition (industrial emissions)	Strong inhibition of root growth, leaf chlorosis, disrupted enzymatic activity, reduced biomass	Reduced flowering and fruiting; developmental abnormalities in seeds	*Lactuca sativa, Arabidopsis thaliana*
Nickel (Ni)	Soil (industrial/mining, sewage sludge)	Inhibited root elongation, chlorosis, reduced biomass, altered leaf morphology	Reduced pollen viability and seed production at higher exposure	*Alyssum spp. (some hyperaccumulators), Phaseolus spp.*
Chromium (Cr, esp. Cr (VI))	Soil and irrigation water (tanneries, industry)	Root damage, reduced germination (if present early), stunted shoots, oxidative stress	Lower flower numbers, reduced seed set and seedling vigor	*Vigna radiata, Brassica spp.*

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Material and Method-

Plant Material and Growth Conditions:

Healthy and uniform seeds of *Andrographis paniculata* (Burm. f.) Wall. ex-Nees were surface-sterilized using 0.1% (w/v) mercuric chloride for 2 min, followed by thorough rinsing with sterile distilled water. Seeds were germinated in plastic trays containing sterilized soil mixture (garden soil: sand: farmyard manure, 2:1:1). After 20 days, uniform seedlings were transplanted into earthen pots (25 cm diameter) containing the same soil mixture and maintained in a greenhouse under controlled conditions (25 ± 2 °C temperature, 60–70% relative humidity, and 14 h photoperiod).

a-*Andrographis paniculata* (control condition)

b- *Andrographis paniculata* (Treated with NaCl)

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c- *Andrographis paniculata* (Treated with $CdCl_2$)

Experimental Design and Stress Treatments:

The experiment was arranged in a completely randomized design (CRD) with three replicates per treatment. After 30 days of growth, plants were subjected to cadmium and sodium stress as follows:

- Cadmium stress: $CdCl_2$ at 0 (control), 25, 50, and 75 µM
- Sodium stress: NaCl at 0 (control), 50, 100, and 150 mM

Stress treatments were applied through irrigation at alternate days for a period of 21 days. Control plants received an equal volume of distilled water. Each pot contained three plants.

Measurement of Growth and Physiological Parameters:

At the end of the stress period, plants were harvested and the following parameters were recorded:

- Plant height (cm) measured from soil surface to shoot apex
- Root and shoot length (cm) measured using a measuring scale
- Fresh and dry biomass (g) determined after oven drying at 70 °C to constant weight

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Relative water content (RWC) was estimated using the standard formula:

$$\text{RWC (\%)} = \frac{FW - DW}{TW - DW} \times 100$$

where FW = fresh weight, TW = turgid weight, and DW = dry weight.

Photosynthetic Pigments:

Total chlorophyll and carotenoid contents were estimated from fresh leaf samples using 80% acetone, following the method of Arnon (1949). Absorbance was recorded at 663, 645, and 480 nm using a UV–Visible spectrophotometer, and pigment concentration was expressed as mg g^{-1} fresh weight.

Biochemical Analysis:

1- Total Soluble Protein

Total soluble protein content was determined according to the method of Lowry et al. (1951) using bovine serum albumin as the standard.

2- Total Carbohydrates

Total carbohydrate content was estimated using the phenol–sulphuric acid method and expressed as mg g^{-1} fresh weight.

Oxidative Stress Markers:

1- Lipid Peroxidation

Lipid peroxidation was estimated by measuring malondialdehyde (MDA) content following the Thio barbituric acid (TBA) reaction method, and results were expressed as nmol MDA g^{-1} fresh weight.

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2- Hydrogen Peroxide (H_2O_2) Content

H_2O_2 content was determined spectrophotometrically using potassium iodide reagent and expressed as µmol g^{-1} fresh weight.

Antioxidant Enzyme Assays:

Fresh leaf tissue (0.5 g) was homogenized in chilled phosphate buffer (pH 7.0) and centrifuged at 12,000 rpm for 20 min at 4 °C. The supernatant was used for enzyme assays.

- Superoxide dismutase (SOD) activity was assayed by its ability to inhibit photochemical reduction of nitro blue tetrazolium (NBT).
- Catalase (CAT) activity was measured by monitoring the decomposition of H_2O_2 at 240 nm.
- Peroxidase (POD) activity was estimated using guaiacol as a substrate.

Enzyme activities were expressed on a protein basis.

Estimation of Secondary Metabolites:

1- Andrographolide Content

Andrographolide was extracted from dried leaf samples using methanol and quantified using High Performance Liquid Chromatography (HPLC) with a C18 column and UV detection at 223 nm. Concentration was expressed as mg g^{-1} dry weight.

2- Total Phenolic Content

Total phenolics were determined using the Folin–Ciocalteu method and expressed as mg gallic acid equivalents (GAE) g^{-1} dry weight.

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Statistical Analysis:

All data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA followed by Duncan's multiple range test (DMRT) at $p \leq 0.05$ to determine significant differences among treatments.

Result and Discussion-

1- Effect of Cadmium and Sodium Stress on Growth Parameters

Cadmium and sodium stress significantly affected the growth performance of *Andrographis paniculata*. A progressive reduction in plant height, root length, shoot length, and total biomass was observed with increasing concentrations of both $CdCl_2$ and NaCl when compared to control plants. However, cadmium stress caused a more pronounced inhibitory effect than sodium stress at comparable treatment levels.

The reduction in growth under cadmium stress may be attributed to Cd-induced inhibition of cell division, disruption of nutrient uptake, and impairment of root system architecture. Sodium stress, on the other hand, likely affected growth through osmotic imbalance and ion toxicity, leading to reduced water uptake and

Table 2- Effect of NaCl and CdCl2 on root and shoot length of *Andrographis paniculata*

Sr.No	Sample Code	Root length (cm)	Shoot length (cm)	Mean ± SD
1	AS1 a	9.5	1.7	1.6 ± 0.10
2	AS1 b	8.5	1.5	
3	AS1 c	8.8	1.6	
4	AHM1 a	8.1	1.2	1.23 ± 0.06
5	AHM1 b	7.6	1.3	
6	AHM1 c	7.5	1.2	
7	Control a	10	2.1	2.03 ± 0.21
8	Control b	11.1	2.2	
9	Control c	9.8	1.8	

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** AS- *Andrographis paniculata* NaCl (S- Salt) ** AHM1- *Andrographis paniculata* CdCl2 (HM- Heavy metal)

metabolic disturbances. Similar growth suppression under heavy metal and salinity stress has been widely reported in medicinal plants and glycophytes.

2. Changes in Relative Water Content and Photosynthetic Pigments

Relative water content (RWC) declined significantly under both stresses, with a sharper decrease under NaCl treatments. This decline reflects impaired water relations caused by osmotic stress under salinity and membrane damage under cadmium exposure.

Total chlorophyll and carotenoid contents showed a concentration-dependent decrease in both stress treatments. Cadmium stress resulted in severe chlorophyll degradation, possibly due to disruption of chloroplast ultrastructure and inhibition of chlorophyll biosynthesis enzymes. Sodium stress-induced chlorophyll loss is attributed to enhanced chlorophyllase activity and reduced synthesis under ionic imbalance. Reduced pigment content ultimately affects photosynthetic efficiency and biomass accumulation.

3. Biochemical Alterations: Protein and Carbohydrate Content

Both Cd and NaCl stress led to a significant decline in total soluble protein and carbohydrate contents in *A. paniculata*. The decrease in protein content under cadmium stress may result from enhanced proteolysis, oxidative modification of proteins, and inhibition of protein synthesis. Under sodium stress, reduced protein levels may be associated with metabolic reallocation toward stress-responsive proteins.

Carbohydrate content declined markedly under stress conditions, possibly due to reduced photosynthetic carbon assimilation and increased utilization of carbohydrates for osmotic adjustment and respiratory energy demands. The reduction was more pronounced under cadmium stress, indicating higher metabolic toxicity.

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4. Oxidative Stress Indicators

Both stress treatments induced oxidative stress in *A. paniculata*, as indicated by significantly elevated malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) levels. Increased MDA content suggests enhanced lipid peroxidation and membrane damage, particularly under higher cadmium concentrations.

Cadmium-induced oxidative stress was more severe than sodium stress, reflecting Cd's ability to disrupt redox homeostasis indirectly by depleting antioxidant pools and interfering with electron transport systems. Sodium stress also increased ROS production, mainly due to osmotic stress and ionic toxicity affecting mitochondrial and chloroplast metabolism.

5. Antioxidant Enzyme Responses

Activities of antioxidant enzymes—superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD)—were significantly enhanced under moderate levels of both cadmium and sodium stress. Increased SOD activity suggests elevated dismutation of superoxide radicals into hydrogen peroxide, while enhanced CAT and POD activities reflect efficient detoxification of H_2O_2.

At higher stress concentrations, a decline or plateau in enzyme activities was observed, particularly under cadmium stress, indicating possible enzyme inhibition or oxidative damage to protein structures. These findings highlight the role of antioxidant defense mechanisms in mitigating stress-induced ROS toxicity, with limited efficiency under severe stress conditions.

6. Modulation of Secondary Metabolites

Cadmium and sodium stress significantly influenced secondary metabolite accumulation in *A. paniculata*. Andrographolide content increased under low to moderate stress levels, particularly under cadmium treatment, suggesting a stress-elicited enhancement of secondary metabolism. This increase may represent an adaptive defense response, as andrographolide exhibits antioxidant and protective properties.

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However, higher stress intensities led to a decline in andrographolide levels, likely due to metabolic inhibition and resource limitation. Total phenolic content followed a similar trend, increasing initially under moderate stress and decreasing at higher concentrations. These results indicate that controlled stress conditions may enhance medicinal value, whereas excessive stress is detrimental.

7. Comparative Effects of Cadmium and Sodium Stress

Overall, cadmium stress exerted a more toxic effect on *A. paniculata* than sodium stress. While both stresses induced oxidative damage and metabolic disturbances, cadmium caused greater growth inhibition, pigment loss, and lipid peroxidation. Sodium stress primarily affected water relations and osmotic balance, with comparatively lower oxidative damage at moderate concentrations.

The differential responses underline distinct stress perception and tolerance mechanisms operating in *A. paniculata* under heavy metal and salinity stress.

8. Adaptive Significance

The coordinated activation of antioxidant enzymes and modulation of secondary metabolites suggest that *A. paniculata* possesses a moderate adaptive capacity to withstand abiotic stress. However, prolonged or high-intensity exposure to cadmium and sodium may compromise plant health and medicinal quality, emphasizing the need for careful cultivation practices in contaminated or saline soils.

Conclusion-

The present study demonstrates that both cadmium and sodium stress significantly influence the physiological and biochemical performance of *Andrographis paniculata*. Exposure to increasing concentrations of $CdCl_2$ and NaCl resulted in marked reductions in growth attributes, relative water content, and photosynthetic pigment levels, indicating impaired plant vitality under abiotic stress conditions. Cadmium stress exerted more severe toxic effects than sodium stress, particularly in terms of growth inhibition, chlorophyll degradation, and oxidative damage.

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Elevated levels of malondialdehyde and hydrogen peroxide under both stresses confirmed the induction of oxidative stress, while enhanced activities of antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase reflected the activation of intrinsic defense mechanisms. However, the decline in antioxidant efficiency at higher stress intensities suggests limited tolerance capacity under prolonged or excessive stress exposure.

Interestingly, moderate levels of cadmium and sodium stress stimulated the accumulation of secondary metabolites, including andrographolide and phenolic compounds, highlighting the role of stress-induced metabolic reprogramming in defense and adaptation. Nevertheless, excessive stress negatively affected metabolite synthesis, potentially compromising the medicinal quality of the plant.

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