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**Research Article**

# IN VITRO ACCUMULATION OF CADMIUM CHLORIDE IN *PAPAYA* SEEDLING AND ITS IMPACT ON PLANT PROTEIN

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**ABSTRACT**

**Background:** *Carica papaya* has wide range of applications in traditional medicine. It has high nutritional content and various medical applications.

**Objectives:** In the present study, changes have been observed at the morphological, biochemical, antioxidation and protein level in *Carica papaya* L variety Pusa Dwarf by accumulation of cadmium chloride *in vitro*.

**Material and methods:** Plants were treated with different concentrations (20ppm, 40ppm, 60ppm and 80ppm) of cadmium chloride, and examined changes in growth and protectively induced oxidative stress in relation to heavy metal in three weeks old seedlings. Protein profiling by SDS-PAGE was done to study the influence of severe heavy metal stress in *Carica papaya* L. variety Pusa Dwarf leaf and root explants.

**Result:** Plant seedlings showed decrease in morphological characteristics like plant height, root and shoot length in response to increasing concentrations of heavy metal stress. Similarly carbohydrate content decreased in both leaves and roots while chlorophyll pigments (a+b) increased in leaf explants. Proline and polyphenolic compounds showed an increase in stressed plants compared to control.

**Conclusions:** Plants undertake many adaptive mechanisms for their survival under metal stress which includes morphological as well as biochemical characters. Proline and polyphenolic compounds indicate the presence of excellent antioxidative ingredients to protect the induced by free radicals. Plant protein profiling supports the effect of heavy metal stress in papaya.

**KEYWORDS:** *Carica papaya*, cadmium chloride, antioxidants, phytochemicals, protein

## INTRODUCTION

*Carica papaya* L. variety Pusa Dwarf belongs to the family Caricaceae is widely cultivated in tropical and subtropical countries. Different parts of papaya including leaves, barks, roots, latex, fruit, flowers and seeds have wide range of applications in traditional medicine [1]. It produces papain, a valuable proteolytic enzyme, and has various medicinal applications. Experiments have shown that *C. papaya* have anthelmintic, antiprotozoan, antibacterial, antifungal, antiviral, antiinflammatory, antihypertensive, hypoglycaemic, hypolipidemic, wound healing, antitumor, free-radical scavenging, antisickling, neuroprotective, diuretic, abortifacient, and antifertility activities [2-4]. Due to the considerable biological activity and medicinal applications, papaya is now considered as a valuable nutraceutical fruit plant. It has low calories and rich in natural minerals and vitamins. It contains vitamin A, C and calcium [5].

In recent years, attention is given to papaya due to the nutritional and medical issues of papaya as its fruit is a good source of carbohydrate, as well as high levels of vitamins (Vitamin C and Vitamin A) and minerals (copper and magnesium) [6]. Papaya contains many biologically active compounds; two important ones are chymopapain and papain, of which papain is a major component of papaya latex and widely applied for meat tenderization. Chymopapain and papain is also believed to aid digestion; varying in amount in the fruit, latex, leaves and roots. The leaves, fruit, stem and roots of papaya yield proteolytic enzyme papain (papayotin), phytokinase, malic acid, calcium maleate and seeds yield volatile oil. Oxidative damage is related to high incidents of some degenerative diseases including cancer, arthritis, arteriosclerosis, inflammation, aging and brain dysfunction. Almost all studies reported, papaya fruits contain low total phenolic compounds. In recent years, papain and other endopeptidases have proven several medical benefits, such as defibrinating wounds and treatment of edemas[7].

Beside the ability to take up essential nutrients, plants absorb and accumulate other metals, even those with unknown metabolic function. The presence of heavy metals in excess amounts is a global problem, threatening the vegetation, wild life and humans [8]. Heavy metal pollution in air, water and agricultural soil is of major ecological concern due to its impact on human health through the food chain and its high persistence in the environment [9]. Plants exposed to stressing agents such as drought, salinity, excess of heavy metals, air pollutants or pathogens have developed strategic defense mechanism that vary between species and the nature of stressing agent.

Cadmium is one of the most important contaminants of the environment. The steel industry and waste incineration followed by volcanic action and zinc production are estimated to account for the largest emissions of atmospheric cadmium [10]. In nature, a very large amount of cadmium is released into the environment, about 25,000 tons a year. About half of this cadmium is released into rivers through weathering of rocks and some cadmium is released into air through forest fires and volcanoes. Rest of the cadmium is released through human activities, such as manufacturing. Uptake of cadmium by human takes place mainly through food. Foodstuffs that are rich in cadmium can greatly increase the cadmium concentration in human bodies. Examples are liver, mushrooms, shellfish, mussels, cocoa powder and dried seaweed. Other high exposures can occur with people who live near hazardous waste sites or factories that release cadmium into the air and people that work in the metal refinery industry. When people breathe in cadmium it can severely damage the lungs. This may cause even death. Cadmium is first transported to the liver through the blood. There, it is bound to proteins to form complexes that are transported to the kidneys. Cadmium accumulates in kidneys, where it damages filtering mechanisms. This causes the excretion of essential proteins and sugars from the body and further kidney damage. It takes a very long time before cadmium that has accumulated in kidneys is excreted from a human body. Proteins are compounds of fundamental importance for all function of cell. Protein variation is an essential part of plant response to abiotic and biotic stress as well as for adaptation to environmental conditions [11].

The aim of this study was to evaluate the response of *Carica papaya* L. variety Pusa Dwarf after the treatment with cadmium chloride for the evaluation of adsorption capacity of cadmium from soil by *Carica papaya* L. variety Pusa Dwarf. The seeds of *Carica papaya* L. variety Pusa Dwarf were used to check the removal or adsorption of cadmium ions from aqueous solution. It was also the intention of this work to compare the biochemical content and protein profiling of plants stressed by different concentration of cadmium chloride. In the present study the adverse effect of cadmium on papaya species of Caricaceae family viz., *Carica papaya* variety Pusa Dwarf, has been chosen to investigate the adaptation mechanism that occur in the plant.

## MATERIALS AND METHODS

### Plant materials and seed treatments

*Carica papaya* L. variety Pusa Dwarf was used to evaluate the physiological and biochemical activities under stress at an early seedling stage. Seeds of the *Carica papaya* L. (variety Pusa Dwarf) were obtained from the Agriculture institute in Jaipur. Seeds were surface sterilized with 0.1% $HgCl_2$ for 5 minutes and washed thoroughly thrice with distilled water. It was soaked in 10% potassium nitrate for half an hour and treated with different concentration of Cadmium chloride (20ppm, 40ppm, 60ppm and 80ppm) for half an hour. Further it was transferred in plastic pots, filled with soilrite mix (a mixture of horticulture grade expanded perlite, Irish Peat moss and exfoliated vermiculite in equal ratio from Keltech Energies Ltd.). Control and three weeks old seedlings of all the concentration of cadmium chloride were collected for further analysis of the study.

### Growth parameters

Growth parameters were determined at the end of stress treatment, shoot length was measured from the soil surface to the newly emerging leaf of the papaya. Root length and entire seedling was measured (cm/plant). The fresh and dry weight of control and treated leaves were measured (g/plant) from randomly selected plants. It is used to evaluate the water status of the leaves.

### Biochemical analysis

Fresh leaves and roots were used for the estimation of soluble sugars. Carbohydrate estimation was done by Anthrone reagent with leaves of three week old seedlings treated with the different concentrations of cadmium chloride. The quantitative estimation of chlorophyll (a + b) was done by using modified Arnon method [12]. Proline estimation was done using ninhydrin [13].

### Phytochemical analysis

Total flavonoid was estimated by using modified aluminium chloride colorimetric method by homogenizing 1g of fresh leaves in 10mL methanol, and centrifuged to obtain a clear supernatant and aliquots were mixed with 95% ethanol, 10% aluminium chloride, 1M potassium acetate and distilled water and the reaction was incubated at room temperature for 30 minutes. The absorbance was measured at 415nm against blank [14].The quantitative estimation of polyphenols was done by using Folin's reagent and 25% sodium. Standard curve was obtained using various concentrations of Gallic acid [15].

### Antioxidant enzyme extraction and assay

The control and treated leaves were excised from the seedlings (0.1g) and homogenized with mortar and pestle at $4^0$C in extraction buffer (50 mM phosphate buffer, pH 7.0). The homogenate was centrifuged at 15000 rpm for 25 minutes. The homogenized was used as the crude extract for the catalase (CAT) enzyme activity[16]. The CAT activity was determined spectrophotometrically by following the decline in $A_{240}$ as $H_2O_2$. Superoxide dismutase activity was estimated by it measuring the inhibition of photochemical reduction of NBT at 560nm [17].

### Protein profiling by SDS PAGE

Protein profiling in the control and cadmium treated plant samples were analyzed by SDS PAGE. The samples of protein were subjected to PAGE [18].

### Statistical Analysis

All the experiments were repeated twice with three replicates (n=3) and data presented are mean ± S.E.

## RESULTS AND DISCUSSION

The current study investigated the physiological, biochemical and proteomic changes in germinating papaya seeds in response to cadmium stress. Leaf extract of three weeks old papaya seedlings treated with various concentrations of cadmium chloride (metal stress) along with its control were used in the present study to investigate the comparative changes at the morphological, biochemical, phytochemical, antioxidant properties and protein level. There was gradual decrease in the plant height, root and shoot length at different increasing concentrations of the cadmium. The results of the present study confirm that metal stress causes a reduction in vegetative growth and decreases in total plant productivity. The exposure of heavy metal $Cd^{2+}$ significantly affected different parameters of papaya such as carbohydrates, chlorophyll (a and b) contents, proline, total polyphenols, flavonoids, antioxidants and total protein contents. The results in relation to the effect of various concentrations of cadmium on the growth performance measured in the term of the morphological changes includes size of leaves, small in treated plants as compared to control. Fresh and dry weight of this plant gradually decreased in treated plant as compare to controlled ones. Overall inhibition was observed in higher concentration of cadmium but the maximum diminution found in 80ppm concentration of three week old treated plants (Table 1). Root and shoot length decreased in case of metal stressed plants (Fig. 1). Effect of metal affects the growth of plant.

**Table 1: Effect of cadmium toxicity on morphological parameters of *C. papaya* L. variety Pusa Dwarf on three weak old seedlings**

| Cadmium concentration in soil (ppm) | Shoot length (cm/ plant) | Root length (cm/ plant) | Leaf fresh weight (g/ plant) | Leaf dry weight (g/ plant) |
|---|---|---|---|---|
| Control | 7.9±0.088 | 5.1±0.057 | 0.294±0.0008 | 0.0203±0.0008 |
| 20 ppm | 7.6±0.057 | 4.56±0.088 | 0.286±0.0016 | 0.022±0.0017 |
| 40 ppm | 7.46±0.176 | 3.93±0.259 | 0.234±0.0061 | 0.018±0.0017 |
| 60 ppm | 7.266±0.12 | 3.7±0.152 | 0.219±0.004 | 0.0138±0.0016 |
| 80 ppm | 7.3±0.23 | 3.46±0.145 | 0.202±0.002 | 0.0117±0.0017 |


**Figure 1: Morphological changes of three weak old seedling of *Carica papaya* L. variety Pusa Dwarf grown under different concentrations of $CdCl_2$**

Biochemical analysis revealed the presence of chlorophyll pigment in the leaves of papaya seedlings under stress condition. Increase in chlorophyll content was observed as compared to control in three weeks stressed plants (Fig 2). Present data obtained from the study of plant material (leaves) extracts of 2.5N HCl, 3% aqueous sulphosalisylic acid and acetone gives the affordable information by showing the presence of carbohydrates, chlorophyll, and proline in the sample of all the concentration of metal stress (Fig. 3). Biomass production depends on the accumulation of carbon products through photosynthesis, but elevated metal stress can adversely affect photosynthesis by altering chlorophyll content. Similar changes in the photosynthetic pigment content by various metal stresses were regarded. Decreased chlorophyll content associated with heavy metal stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis. Cadmium affects chlorophyll biosynthesis and inhibits protochlorophyll reductase and aminolevulinic acid (ALA) synthesis. But present results showed that chlorophyll contents were increased in stress condition as compare to control. The chlorophyll ratio, which is used as a stress indicator, increased slightly with increasing metal treatments.

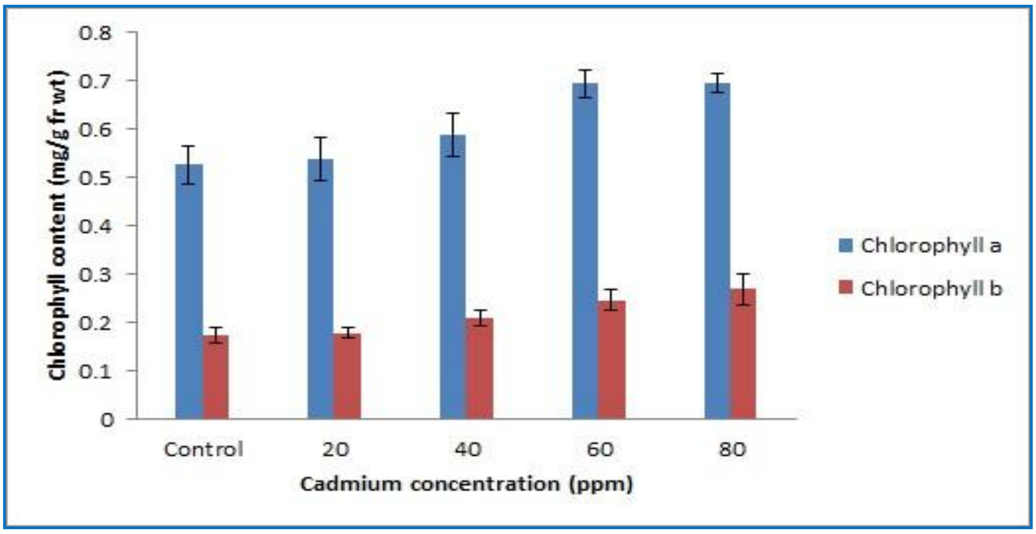



**Figure 2: Effect of cadmium toxicity on photosynthetic pigments**

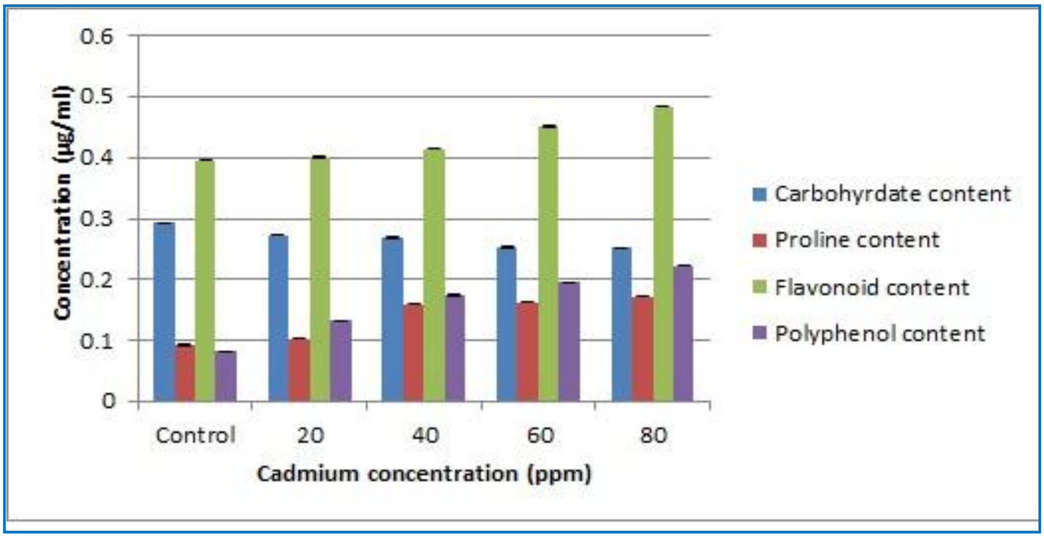



**Figure 3: Biochemical contents of *Carica papaya* L. variety Pusa Dwarf on three weeks old plant**

Soluble carbohydrate content in plants decreased with increasing concentration of heavy metals. Carbohydrate amount in papaya leaf is more but it has been found that in concentration (80ppm) of $CdCl_2$ the carbohydrate concentration was decreased in leaf explants of three weeks old seedlings as compare to control. Increase in the osmoprotectant proline content was directly proportional to the heavy metal i.e., cadmium chloride concentration. It has been observed that proline content was more in 80ppm concentration of cadmium chloride in three weeks old seedlings as compare to control.

The phytochemical analysis revealed the presence of flavonoids and polyphenols. The flavonoid content is gradually increasing with the increasing concentrations of metal toxicity. Increasing polyphenols and flavonoids explains the defense mechanism of this plant. It is clear from the results that as the time exposure with cadmium concentration increases polyphenols increases i.e. in three weeks old treated plants is higher in concentration as compare to control. It is clear that increasing polyphenols in stressed leaf explants known to support bioactive activities and thus responsible for the antioxidant activities of this plant. Similarly the polyphenol content was also increased in the stressed seedlings in all concentration of $CdCl_2$ as compared with control. Flavonoid content was increased in the stressed seedlings in all concentration of $CdCl_2$ compare to control.

Maximum antioxidant activity was found in treated plants with $CdCl_2$ toxicity. Antioxidant property reduced when the plants were exposed to increasing concentration like 60ppm and 80ppm of $CdCl_2$. It is clear from the data that 40ppm concentration of three weeks old seedlings has responded in increasing anti oxidative property. The enzymes Superoxide Dismutase (SOD) were increased in the leaves as compare to control. These results confirmed the fact that SOD plays an important role to alleviate oxidative stress by scavenging reactive oxygen species from cell compartment. It has been noticed that with the increase in the concentration of cadmium, Catalase activity was correspondingly decreased (Fig. 3 and 4).

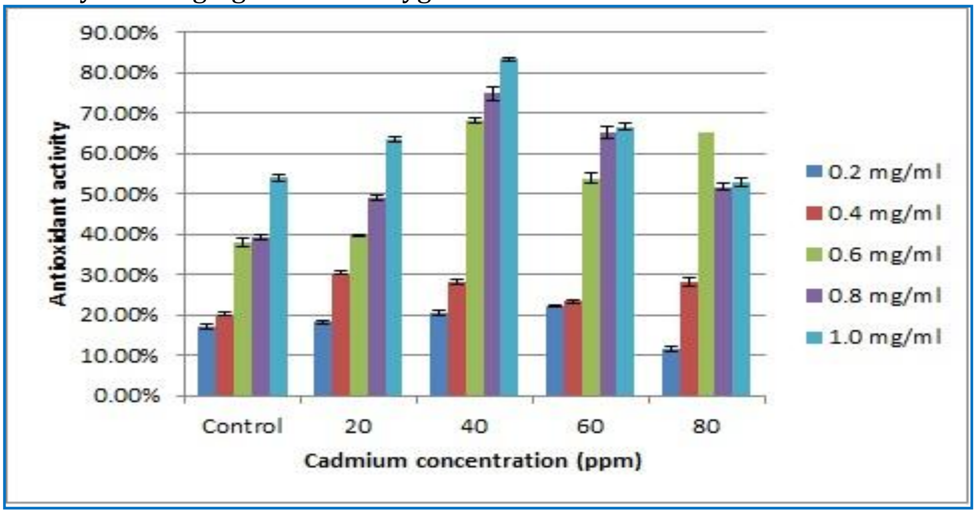
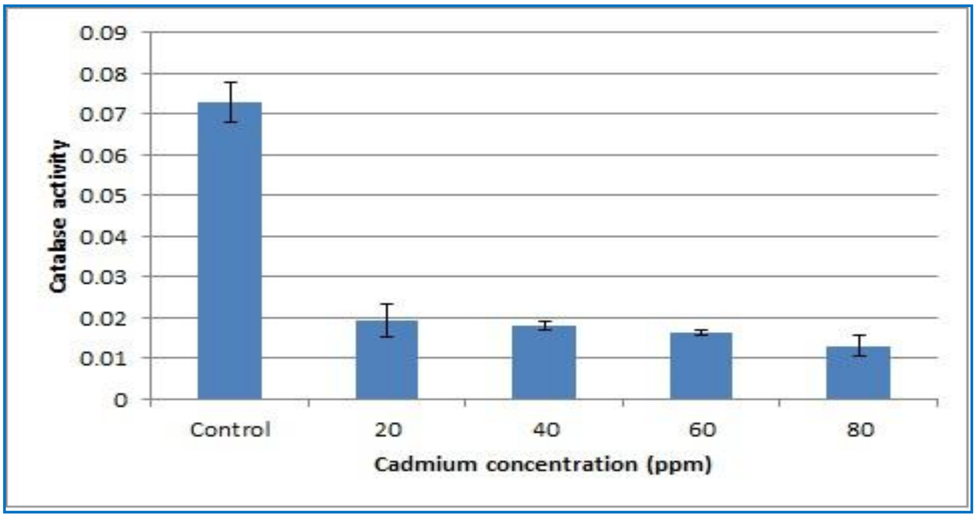



**Figure 4: Antioxidant activities of methanolic extract of three weeks old papaya seedlings**



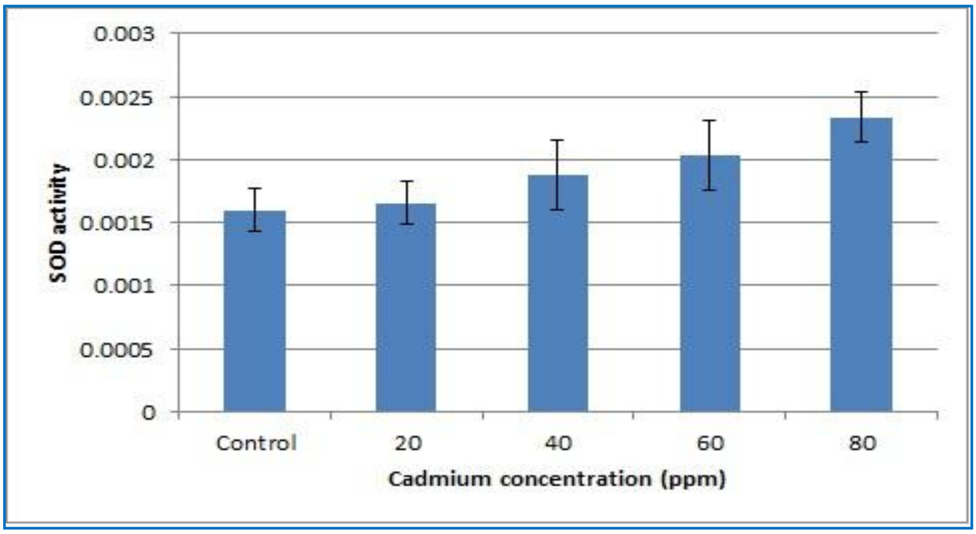



**Figure 5: Effect of cadmium toxicity on antioxidant enzymes of *Carica papaya* L. variety Pusa Dwarf of three weeks old plant**

Protein contents in the leaves were seen at the molecular level. Toxicity of cadmium altered the protein content of leaves. It has been observed that protein was increased with increasing time exposure in various concentration of cadmium like three weeks old seedlings exposed with 20, 40, 60 and 80ppm concentration of cadmium. To investigate the effect of cadmium on the protein patterns of germinating papaya seeds, total proteins from three weeks old seedlings of leaf of papaya in different concentrations of cadmium chloride were extracted and subjected to one-dimensional SDS-PAGE. In this study the content of soluble protein was increased by cadmium stress up to certain limit then slightly declined. The disappearance of some proteins in response to cadmium stress indicates that such treatments are effective in causing a major re-shuffle of protein profile of plant. New bands compare to control may have a role in the mechanism of cadmium tolerance that enables the plant to cope with stress conditions. Few more bands of polypeptides in treated plants are observed than standard and control. We assume that the differences between the polypeptide profiles reflect some structural difference between the control and those of the treated plant seedlings of various age groups with different concentration of cadmium toxicity.

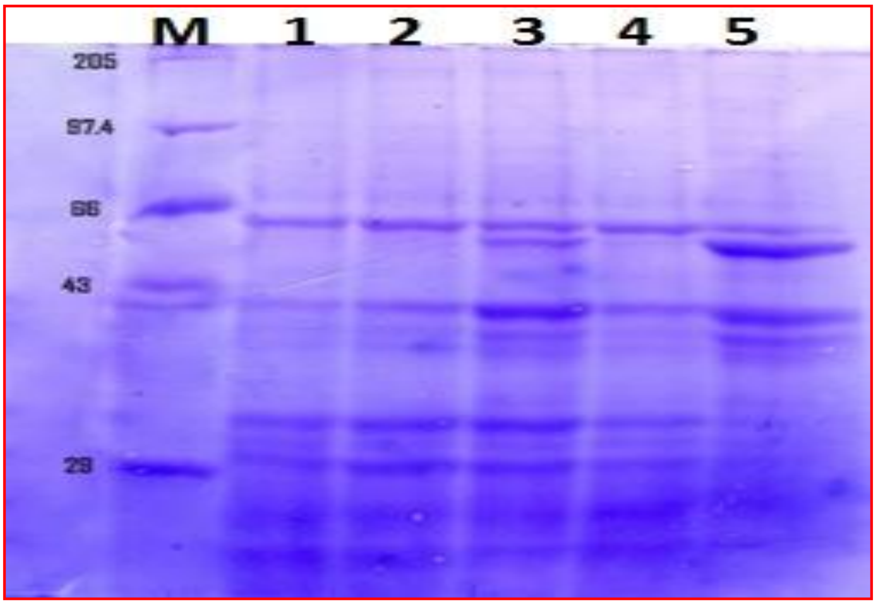



**Figure 6: SDS-Page bands of proteins obtained from control and cadmium chloride treated seedlings at three-week-old seedlings. In these the M is for marker and series 1-5 represents control,20ppm,40ppm,60ppm,80ppm cadmium chloride stressed leaves respectively**

The proteins were resolved into distinct bands that spanned a broad range of apparent molecular weight from 29kDa to more than 205kDa. Some visible differences in the protein band patterns were observed between standard, control and cadmium treated samples (Fig. 5). Increasing cadmium concentrations produced significant changes in protein patterns of the germinating papaya seeds. It is clear that polypeptide band in lane3 and lane 5 in between 66kDa- 43kDa has some specificity. It is very clear from the figure 2 that as concentration of toxicity increases, the prominence of polypeptide bands is clear and three weeks old seedling in 80ppm polypeptide bands slightly diminish in the gel in between 1kDa-29kDa of standard protein marker.

## CONCLUSION

In the present work, *Carica papaya* L. variety Pusa Dwarf was exposed to various concentration of cadmium metal and its biochemical factors, phytochemicals, antioxidant factors and protein contents were studied. The toxicity of the cadmium metal was investigated and results were tabulated. In response to heavy metal stress, in the plants seedlings the morphological characters like leaf/root color, size, length and number of leaf/root were found to be decreased as compare to control. While chlorophyll (a+b) was increased as concentration of heavy metal increased with increasing exposure time. The increase in $Cd^{2+}$ concentration in papaya seedlings caused significantly proline accumulation and also increased flavonoid and polyphenols increased in stressed leaf explants of plant as compare to control. We found that leaf explants of papaya seedlings have free radical scavenging activity and major active components are polyphenols. The leaves of papaya might be an excellent antioxidant ingredient to protect damage induced by free radicals. Soluble protein contents were also increased in stressed plant's seedling. But few new polypeptides bands were observed that has to be used for further detail study. Plants undertake many adaptive mechanisms for their survival under metal stress which includes morphological as well as biochemical characters. It could be thus concluded that biochemical tolerance to $Cd^{2+}$ toxicity is related to the capacity of plant to activation of antioxidant defense system and accumulation of proline a universal protectant of various stress, may be used for phytoremediation.

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