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HEPATOPROTECTIVE ACTIVITY OF *OPUNTIA DILLENII* (KER GAWL.) HAW. FRUIT PULP EXTRACT AGAINST CADMIUM INDUCED TOXICITY IN MICE

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ABSTRACT

The present study was conducted to evaluate the hepatoprotective activity of *Opuntia dillenii* (Ker Gawl.) Haw. (OD) fruit extract in mice (*Mus musculus*) against Cadmium (Cd) induced hepatic-histopathology. Thirty albino mice were randomly distributed in three groups (n=10). Control(C) (untreated group) maintained on regular supply of food and drinking water. The Cd and Cd+OD groups were given 50ppm Cd ions in drinking water for 15 days and Cd free water for the next 7 days. The Cd+OD group additionally received 0.2ml of OD fruit extract (once daily) by gavage on days 16-22. The histological slides in Cd group revealed scattered aggregations of hepatocytic debris in the wavy hepatic cords with little sinusoidal spaces in between. Mostly the hepatocytes were swollen containing enlarged nuclei and vacuolated cytoplasts. In Cd+OD group these pathological signs were partly reversed additionally scattered aggregations of hepatoblastic progenitor cells were also visible. Mean cross-sectional areas of central lobular vein, hepatocyte and hepatocytic nucleus were significantly ($p \leq 0.05$) higher in Cd ($6368.34 \pm 5.87 \mu^2$, $400.9 \pm 20.01 \mu^2$ and $82.64 \pm 9.07 \mu^2$ respectively) than the control ($3280.20 \pm 1.83 \mu^2$, $184.10 \pm 11.77 \mu^2$ and $65.86 \pm 7.1 \mu^2$) and Cd+OD ($3824.64 \pm 2.17 \mu^2$, $389.51 \pm 26.74 \mu^2$ and $76.14 \pm 6.06 \mu^2$) groups whereas, the mean number of hepatocytes per hepatic cord were significantly ($p \leq 0.05$) higher in control (11.60±.37) than Cd+OD (10.84±.43) and Cd (8.56±.32) groups; however the mean number of progenitor cells perunit area were significantly higher in Cd+OD (6.76±.26) than control (4.43±.23) and Cd (2.32±.21)group. These findings reflect the curative role of the *O. dillenii* fruit extract on the hepato- histopathological changes of Cd exposure in mice, clearly indicating its hepato-protective medicinal importance for similar possible human benefits.

Key words: *Opuntia dillenii*, Cadmium toxicity, Hepatohistopathology, Mianwali.

INTRODUCTION

Cadmium is an environmental pollutant and cause toxic effect to nearly every system in the animal body (Patra *et al*., 2011). Cadmium causes severe damage in various organs such as kidney, liver, testis and ovaries in both humans and animals (Thompson and Bannigan, 2008; Liu *et al*., 2010). Increases in the level of Cd toxicity leads to the production of reactive oxygen species which cause damage at cellular level (Namjooyan *et al*., 2012). Physiological and metabolic processes are disturbed due to oxidative stress produced by reactive oxygen species (Schutzendubel and Polle, 2002). Increased generation of ROS decreases the antioxidant defense systems (Datta *et al*., 2000). Liver is the vital organ of the body. Liver is involved in regulation of homeostasis in the body in all the biological processes related to growth, fight against disease, nutrient supply, energy production and reproduction (Rajkapoor *et al*., 2008). Any hepatic damage due to environmental toxins, heavy metals, poor eating habits, alcohol and drug use leads to the distortion of metabolic functions of liver (DelRaso *et al*., 2003). Many synthetic drugs used for the treatment of hepatic ailments also damage the liver (Saleem *et al*., 2008). Plant extracts are advised for treatment of liver diseases due to the presence of natural antioxidants (Latha and Reddy, 2012; Costa *et al*., 2013). It has also been observed that antioxidant prevents Cd induced hepatic toxicity (Sheikh *et al*., 1999). A number of plants have been used in the traditional medicines for the treatment of liver diseases (Rai, 1994; Schuppan *et al*., 1999; Das *et al*., 2012).

Opuntia dillenii (Ker Gawl.) Haw. (Fig. 1) belongs to family Cactaceae, commonly known as prickly pear or pear bush. Cactus plant is a xerophyte succulent shrub in semi desert regions in dry sunny areas. *Opuntia dillenii* plant contains excess amount of nutrients, such as phenolic compounds, polysaccharides, minerals, betalains, organic acids, lipids, vitamins and amino acids. Its purplish fruit is used as a colouring agent in foods, ice cream and drinks (Chang *et al*., 2008). Traditionally *Opuntia* species are used as source of medicines for the cure of diseases like gonorrhea, gastrointestinal problems and inflammatory lesions (Ahmed *et al*., 2005). Cactus pear (*Opuntia dillenii*) has antioxidant activities that considerably decrease oxidative stress in patients against chronic pathologies. Betalain pigments found in cactus

pears have sound effects in cell growth and inflammation (Siriwardhana *et al*., 2006). It has been reported that that the total antioxidant action of the cactus pear is due to the presence of vitamin C, polyphenols, flavonoid compounds, pigments, betalains and taurine (Castellar *et al*., 2003). Flavonoids are powerful antioxidants in vitro. Among plant phenols, flavonoids are strong ROS scavengers (Rice-Evans, 2001; Tattini *et al.,* 2004). Several secondary metabolites occur in *O. dillenii* and they enhance the antioxidant activity of the plant. In fruits of *O. dillenii* antioxidants are found in excess quantity (Qiu *et al*., 2002). Betacyanins, betaxanthin, flavoineds and ascorbic acid are present in purple colour fruit extract of cactus pear show highest antioxidant capacity (Chang *et al*., 2008).

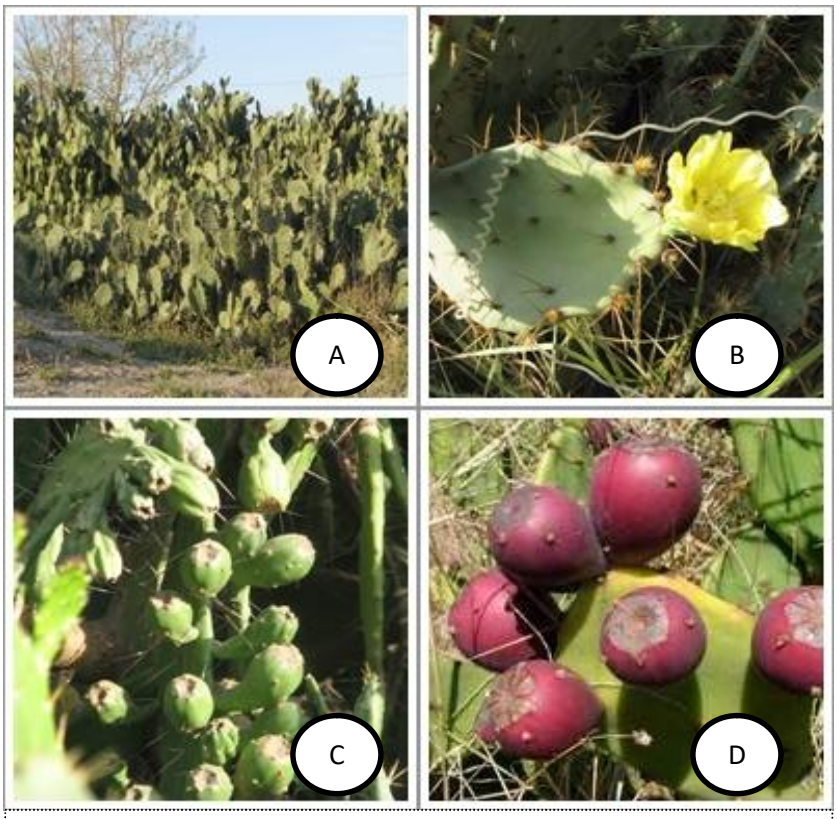

Fig. 1. *Opuntia dillenii* (Ker Gawl.) Haw. a. vegetation, b. closer view of stem bearing flower, c. immature fruits, d. ripe fruits

The aim of the present study was to discover the hepato-protective role of *O. dillenii* fruit extract against Cd induced oxidative stress in mice liver.

MATERIALS AND METHODS

Experimental animals and their maintenance: Thirty adult male albino mice (*Mus musculus*) were used in the present research work. The animals were reared under standard conditions in the animal house of the Department of Biological Sciences, University of Sargodha, Sargodha, Pakistan. They were kept under standard housing conditions. Free access to standard rodent diet enriched with vitamins and dried milk and drinking water was provided to the animals throughout the study period.

Dose groups.

Control (untreated): These animals were given normal diet and regular drinking water.

Cd group: These animals were given 50ppm Cd ions in drinking water for 15 days along with normal food followed by Cd free water for next 7 days.

Cd+OD group: These animals were given Cd treatment as in Cd group for 15 days while 0.2ml of *O. dillenii* fruit extract was given to them in next 7 days on 12 hourly bases.

Dose preparation

Preparation of Cd dose: A stock solution of 1000ppm Cd ions was prepared by dissolving 1.79g of $CdCl_2$ (laboratory grade) in 1 Liter of water. The required

strength (50ppm) was then prepared (for animal exposure) by diluting 5ml stock solution with 950ml of water.

Preparation of *O. dillenii* fruit extract dose: Fruits of *O. dillenii* were collected from the area of Tari Khel in district Mianwali during the month of December 2013. Spines were removed from the fruit carefully and juice was obtained by pressing the fruit. The crude extract obtained was centrifuged at 500 rpm for 15 minutes and purple supernatant was separated. For experimental use this extract was stored at -30^{0}C in eppendorf tubes and freshly thawed extract was used for animal treatment.

Organs recovery: One the 23rd day of experiment all animals were dissected for surgical removal of the liver. Organs were weighted just after the dissection and were fixed in Carnoy's fixative for histological processing.

Processing and staining: Liver tissues from all groups were processed in series of steps for wax embedding. Different grades of alcohol were used for dehydration. Dehydrated organs were placed for 1-2hrs in xylene for clearance. Finally, they were placed for 3-5 hours in molten histological paraffin wax for embedding. Blocks of these paraffinized organs were prepared in rectangular glass cavities. Serial sections (5µ) were obtained on a rotary microtome and stretched on albumenized histological glass slides. These serial cross sections were stained with Hematoxyline and Eosin dyes and mounted in Canada balsam for further studies and record.

Digital photography and processing: Photomicrographs of the selected histological sections of the liver of experimental groups (Control, Cd and Cd+OD) were obtained using Sony (Model no. DSC-W35) 7.2 mega pixel digital camera mechanically fitted on a Labomed CXR2 trinocular microscope. Photographs were processed in CorelDRAW11 for presentation purpose (Fig. 2).

Micrometry: The micrometric data was generated through digital measurements of the photomicrographs of the histological sections in CorelDRAW11.Ten of each animal randomly selected section from each group were used for the measurements of CSA of hepatocytes and their nuclei and the centri-lobular veins. The data obtained was used to obtain mean values in such a way that each animal represented as a unit. To obtain various CSAs diameters were obtained with the help of right-angle perpendicular lines drawn across images of cells passing through the center. The calibrated values were put in the following formula to calculate CSA: CSA= (Length × Width/4) π**.**

Statistical Application: Data based on the micrometry was analyzed through SPSS software for ANOVA and Duncan's Multiple Range Test and presented in the form of bar graphs in the result section.

RESULTS

Histological results: The histological slides in control group revealed all the signs of healthy liver microanatomy that include distribution of the hepatic layout in the form of lobules containing radiating hepatic cords with centrally located lobular veins. The hepatic cords were in straight queues of one cell thick hepatocytes lined with kupffer cells on both sides. The adjacent hepatic cords were separated by the hepatic sinusoids of almost uniform diameter. Bi-nucleate hepatocytes were frequently seen in the hepatic cords (Fig2 A). In Cd group the hepatic cords were misaligned and hepatocytes were swollen resulting into almost complete obliteration of the sinusoidal spaces. The nuclei of the hepatocytes were enlarged while the cytoplasm contained vacuolations. The kupffer cells and bi-nucleated hepatocytes were rarely visible. Apoptotic signs in terms of hepatocytic vacuolations with simultaneous nuclear disintegration and the presence of cellular debris were seen at some isolated places near the centri-lobular vein (Fig. 2B). In Cd+OD group slides the hepatic lobules showed various signs of hepato-lobular regeneration (presence of various aggregations of small oval progenitor cells and juvenile mono and bi-nucleated hepatocytes- not properly arranged into hepatic cords, with centrally placed compact rounded nuclei and cytoplasm showing no signs of vacuolations). Whereas some relict signs comparable to Cd group (presence of cellular debris at various isolated places in the lobules and a few hepatocytes with enlarged nuclei and cytoplasmic vacuolations) were also seen. However, the most interesting finding in this group was localized clumps of small undifferentiated hepatoblastic progenitor cells. Additionally, the progenitor cells were also seen diffused among the intoxicated hepatocytes (Fig.2 C).

Micrometric results

Mean CSA of centri-lobular vein: Highest Mean CSA of central centri-lobular vein was recorded in C (6368.34±5.87µ^2), followed by Cd+OD (3824.64±2.17µ^2) and Cd (3280.20±1.83µ^2) groups. Analysis of variance showed highly significant variation among the groups (P≤0.0001); whereas post hoc analysis had shown that the control group differed significantly (P≤0.05) with other two groups (Fig. 3).

Mean number of hepatocytes per unit area (6400µ^2): Highest mean number of hepatocytes were recorded in C (11.60±.37), followed by Cd+OD (10.84±.43) and Cd (8.56±.32). Analysis of variance of the data showed highly significant variation among the groups (P≤0.0001) whereas post hoc analysis indicated significant (P≤0.05) difference of Cd with the other two groups (Fig. 4).

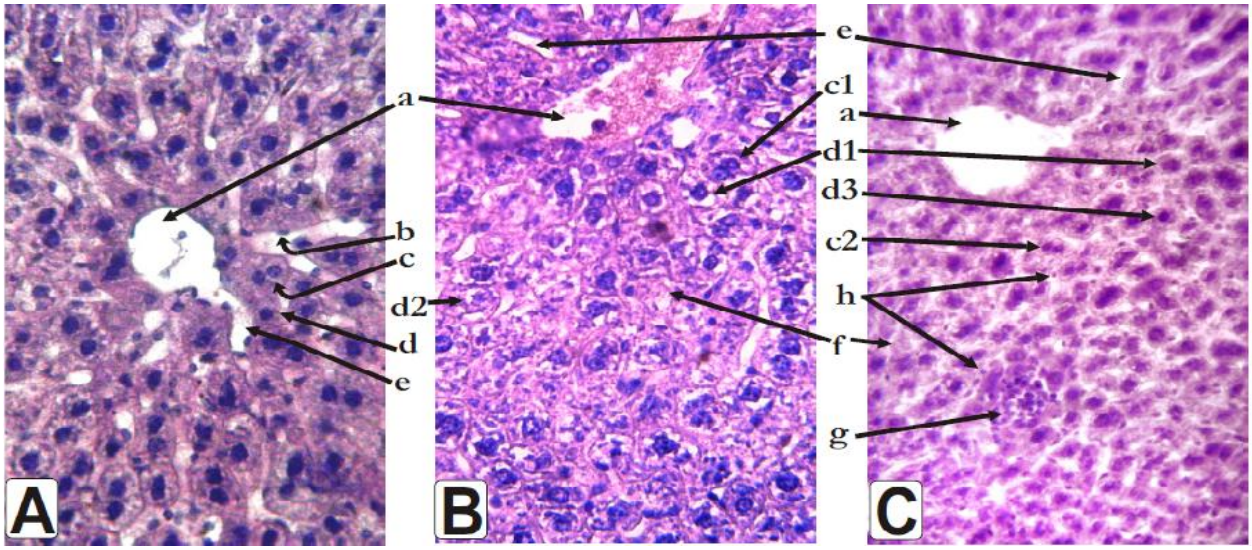

Fig. 2: Selected section of liver from A: Control, B: Cd, C: Cd+OD groups. [a: Centri-lobular vein, b: Kupffer cell c: Mature bi-nucleate hepatocyte, c1: Vacuolated bi-nucleate hepatocyte c2: Juvenile bi-nucleated hepatocyte d: Mature mono-nucleate hepatocyte, d1: Vacuolated mono-nucleated hepatocyte d2: Mono-nucleated hepatocyte under nuclear disintegration and apoptosis d3: Juvenile mono-nucleated hepatocyte, e: Sinusoids, f: Apoptotic cell debris, g: Clumped mass of hepato-blastic progenitor cells, h: Floating hepato-blastic progenitor cells].

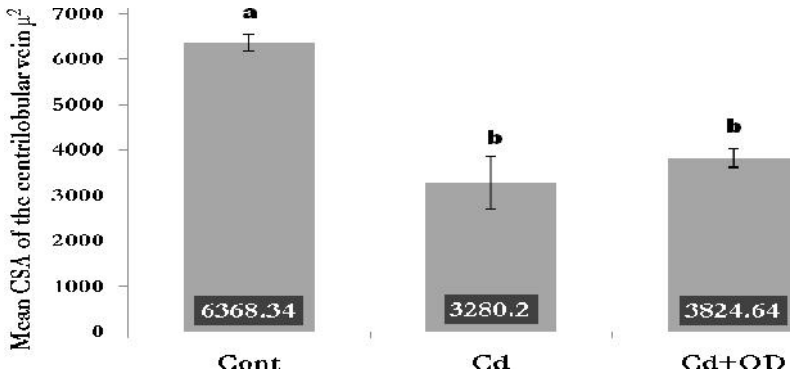

Fig. 3. Mean CSA of centri-lobular vein in C, Cd and Cd+OD groups, ± bar indicates SEM (any two groups not sharing a common lowercase letter differ significantly (P ≤ 0.05) with each other).

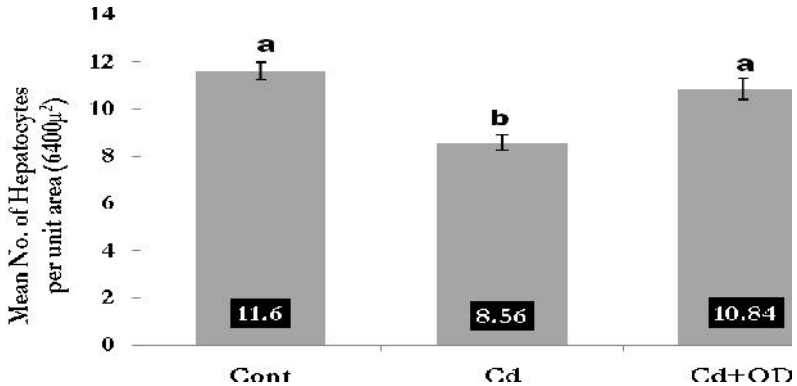

Fig. 4. Mean number of hepatocytes per unit area (6400µ²) in C, Cd and Cd+OD groups, ± bar indicates SEM (any two groups not sharing a common lowercase letter differ significantly (P ≤ 0.05) with each other).

Mean CSA of hepatocytes: Highest Mean values of CSA of hepatocytes were recorded in Cd (400.9±20.01μ^2), followed by Cd+OD (389.51±26.74μ^2) and C (184.1±11.77μ^2). Analysis of variance shows highly significant variation among the groups (P≤0.0001). The post hoc analysis indicated that both Cd and Cd+OD groups differed significantly (P≤0.05) with the control (Fig. 5).

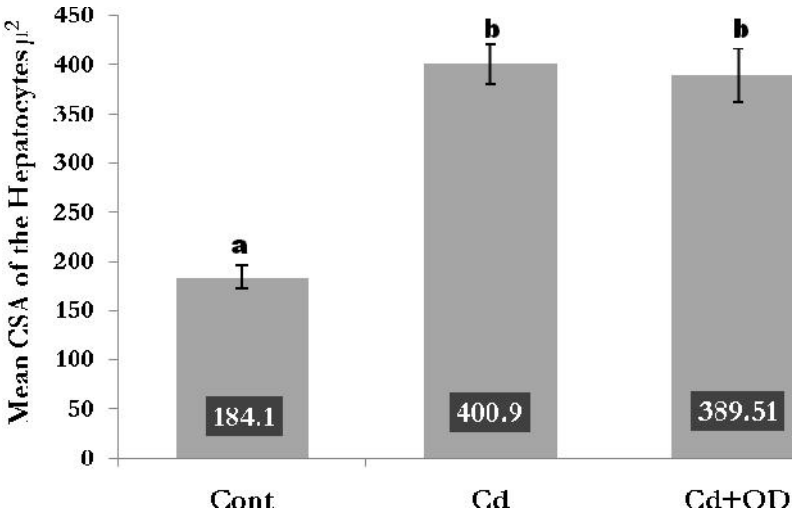

Fig. 5. Mean CSA of hepatocytes in C, Cd and Cd+OD groups, ± bar indicate SEM (any two groups not sharing a common lowercase letter differ significantly (P ≤ 0.05) with each other).

Mean CSA of hepatocytic nuclei: Highest Mean values for CSA of hepatocytic nuclei were recorded in Cd (82.64±9.07μ^2), followed by Cd+OD (76.14±6.06μ^2) and Control (65.86±7.1μ^2). Analysis of variance showed no significant variation among the groups, however post hoc analysis showed significant difference (P≤0.05) between Control and Cd groups (Fig. 6).

Mean number of progenitor cells per unit area (6400μ^2): Highest Mean number of hepatoblastic progenitor cell per unit area were recorded in Cd+OD (6.76±.26) followed by Cd (4.43±.23) and C (2.32±.21) groups. Analysis of variance showed significant variation (P≤0.05) among the groups, whereas all the three groups showed significant difference (P≤0.05) with each on post hoc analysis (Fig. 7).

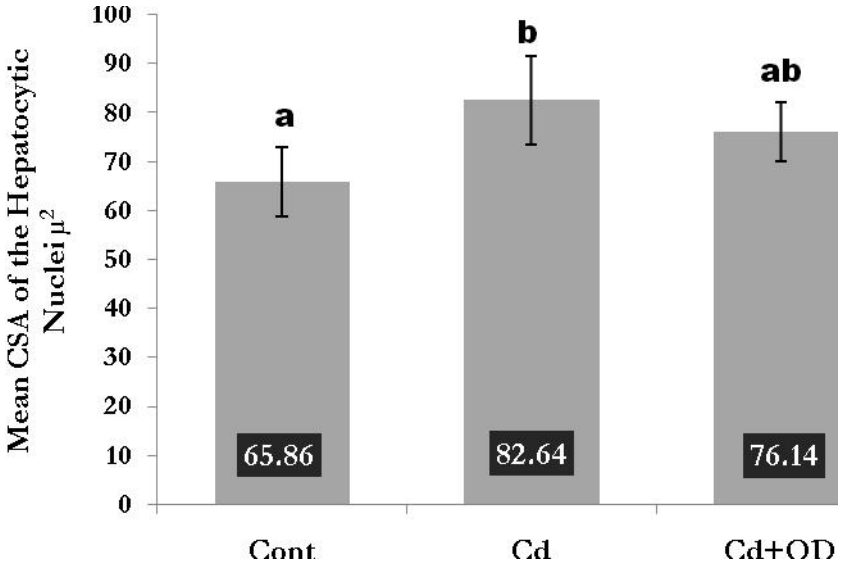

Fig. 6. Mean CSA of hepatocyte nuclei in C, Cd and Cd+OD groups, ± bar indicates SEM (any two groups not sharing a common lowercase letter differ significantly (P ≤ 0.05) with each other).

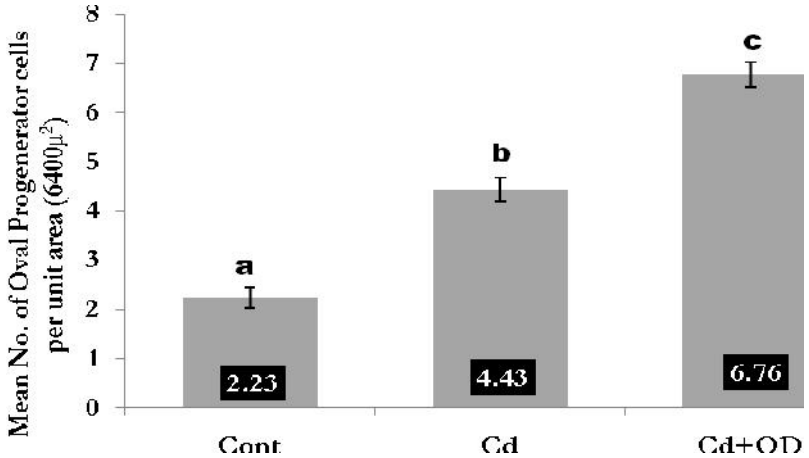

Fig. 7. Mean number of oval cells in C, Cd and Cd+O D groups ±bar indicates SEM (any two groups not sharing a common lowercase letter differ significantly (P ≤ 0.05) with each other)

DISCUSSION

Liver is the organ meant for homoeostatic regulations, storage and controlled release of metabolites. It has key role in detoxification of noxious environmental chemicals (Ahsan *et al*., 2009). In the process of detoxification, the environmental toxicants have been reported to induce various pathological changes in the liver that are expressed in terms of certain biochemical and archeitectural changes in the hepatolobular structures. Gene products can bring about oxidative damage in target organs (Block *et al*., 2002). Cadmium is such an environmental toxicant that has injurious effect on the liver and damages the normal functioning of other body organs (Stohs *et al*., 2001). In this connection Hyder *et al* (2013) has reported that chronic Cd exposure has caused hepatic inflammation leading to necrosis, non-alcoholic fatty liver, non-alcoholic steatotic-hepatitis and hepatic failure causing death in US. Liu *et al.*, (2010) experimentally proved that Cadmium increases in uterine wet weight in rodents. In present study hepato-histopathology of Cd exposure and their ameliorations on OD treatment are reported. The results showed characteristic histological (hepatocytic nuclear enlargements and cytoplasmic vacuolations, presence of hepatocytic debris, misalignment of hepatic cords with a simultaneous obstruction of the sinusoidal spaces) and micrometric alterations (significant decrease in the central hepatolobular vein, number of hepatocytes per-unit area with simultaneous increase in the mean cross-sectional area of the hepatocytes and hepatocytic nuclei) in exposed mice liver as compared to control. These findings clearly suggest that Cd exposure had led to intracellular hepatocytic water accumulation (intracellular edema as reflected by the vacuolations in the cytoplasm) leading to increase in the hepatocytic CSA that in turn caused misalignments of the hepatic cords and compression of the sinusoidal spaces, thereby blocking flow of blood from the marginal blood vessels to the centrilobular vein and thus the CSA of the centrilobular vein was significantly decreased in this group as compared to the control. Significant increase in the mean nuclear CSA of the hepatocytes indicates probable DNA damage due to Cd exposure. In this context it has already been reported that Cd forms DNA adducts by binding with DNA strands, which effect DNA repair and nuclear size increase (Filipic and Hei, 2004). The DNA damage has been considered as the start point of apoptosis (Chandra *et al*., 2000). Thus the apoptotic hepatocytes seen in the current study were probably the logical end result of DNA damage on Cd exposure. In Cd+OD group many cytological signs of recovery and regeneration in the liver were seen these include structural parameters of hepatocytes and hepatolobular dimensions (cytoplasmic and nuclear dispositions of the hepatocytes, centrilobular veins and the appearance of regenerative heptoblastic progenitor cells). Studies revealed that various plants contain immense healing and regenerative medicinal potentials on the diseased animal and human tissues (Das, 2013). One possible mechanism of such rehabilitative and regenerative potentials may the alleviation of the oxidative stress and lipid peroxidation. These capacities of the medicinal plants are attributed to their precious phytochemical antioxidant contents that mainly include flavanoids (quercetin, kaempferol and isorhamnetin) and polyphenoles. Secondary metabolites like Flavonoids that are found in many angiosperms confer a variety of biological functions (Gould and Blister, 2006). The phenolic compounds can potentially delay pro-oxidative effects on proteins, lipids and DNA mainly through generating stable radicals (Tesoriere *et al*., 2005). Pourahmad *et al.*, (2003) reported that severe chronic hepatic disease result from the hepatic accumulation of Cu, Ni, Co or Fe in humans and on the other hand Cd,

Cr_2O_7 and As may induce Lung or Kidney cancer. Opuntia fruit extract is also known for such phytochemicals with strong anti-oxidative capacity (Hatem *et al*., 2010). The resultant histological signs of recovery and regeneration of liver on Opuntia fruit extract treatment in Cd exposed mice indicate its unique hepato-protective and regenerative ability. This exceptional potential of Opuntia fruit extract must be attributable to its unique phytochemical constituents such as the flavonoids (Baek *et al*., 1996), triterpenoids (Xiong *et al*., 2003), saponins (Tran *et al*., 2001) and alkaloids (Vijayan *et al*., 2003).

Conclusion: Based upon the results it was concluded that *O. dillenii* fruit extract possess hepato-protective and hepato-lobular regenerative potentials. Thus, it should be further investigated for possible medicinal alternative in various infectious and toxicological injuries to the liver in humans.

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