Agrian-wild *Opuntia dillenii* Fruit Extract can Ameliorative Cd Induced Hepatic Anomalies

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Abstract

Wild *Opuntia dillenii*, a spiny pest pear, is a cactus native to tropical and subtropical deserts and semi-desert land. It is completely ignored by farmers and researchers due to its prickly and spiny nature, but its flowers, reddish-purple fruit, and latex have potent medicinal values. The present research article evaluates the ameliorative role of *Opuntia dillenii* fruit extract (OFE) against cadmium (Cd) induced hepatic anomalies in mice. Thirty albino mice (*Mus musculus*) were randomly equally grouped as C; Control, Cd; 50ppm Cd in drinking water for 15 days after followed withdrawal and Cd+OFE; (Cd +*Opuntia dillenii*) treated as Cd but additionally post-treatment by 0.2ml/12h OFE for next 7 days. Recoveries were made on the 23rd day of the experiment, and the liver was separated, weighed, and processed for further histopathological studies. Cd treated has irregular hepatocytes with large nuclei, steatosis and apoptotic cells with debris make constricted sinusoid spaces. There was a significant reduction of the number per unit area and cross-sectional area (CSA) of the hepatocytes with elevated ($P<0.05$) CSA of the central lobular vein. These pathological signs were convincingly recovered and regenerating hepatoblasts potential was dominant in the Cd+OFE group indicating the rescuing potential. These findings indicate the hepatoprotective role of the *O.dillenii* fruit extract on the hepatohistopathological changes of Cd exposure indicating their hepatoprotective medicinal importance for similar possible human benefits.

Keywords: ameliorative, hepatic anomalies, hepatocytes, histopathological, hepatoblast

Abbreviations: OFE; *Opuntia dillenii* fruit extract, Cd; cadmium, CSA; sectional area

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Introduction

These metals are considered as environmental pollutants; and become part of the food chain as they are taken at the primary producer level and then used at the consumer level (Dey et al., 2007). Their high concentration knocks off the balance of the ecosystem because once they accumulate in bodies, they cause toxic effects on biota and even death in most living organisms (Gupta, 2013).

One such toxicant is cadmium (Cd) which affects calcium, phosphorous, and bone metabolism in people exposed to Cd in general environment (Jarup, 2009) similarly renal damage is reported in workers exposed to welding metals and Ni-Cd battery factory (Zhang et al., 2008). The use of phosphate fertilizers can increase the toxic effect of Cd in food products (Sheppard et al., 2009). In the human body, Cd accumulates in the body as about 30 mg at the age of 50. Cd causes toxic effects on nearly every system in the animal body (Patra et al., 2011). Cd is the most important toxic chemical due to its increasing level in the environment as outcome of industrial and agricultural practices (Othman et al., 2011).

Various pathological conditions are associated with Cd accumulation in human organs (Arisawa et al., 2007), causing severe damage in various organs such as kidneys, liver, testis, and ovaries (Liu et al., 2010). Cadmium chloride causes a reduction in the weights of testes, seminal vesicles, and epididymis. Increases in the level of Cd toxicity lead to the production of reactive oxygen species which cause damage at cellular level (Namjooyan et al., 2012).

These reactive oxygen species disturb balance by increasing lipid peroxidation, excretion of urinary lipid metabolites, DNA and cell membrane damage, and destruction of cell constituents (Lushchak, 2011). Oxidative stress in body organs is due to any harmful drug or toxic level of metals. In the case of oxidative stress, various free radicals are generated in the extracellular and intracellular environment and their production increases the onset of degenerative diseases. Heavy metals produce ROS resulting in hepatotoxicity, neurotoxicity, and nephrotoxicity in humans and animals. Lipid peroxidation is the principal mechanism for Cd-induced toxicity. The liver is involved in regulating homeostasis in the body in all the biochemical reactions related to growth, the fight against disease, nutrient supply, energy production, and reproduction. detoxification the liver can detoxify xenobiotics and antibiotics.

Many synthetic drugs are used for the treatment of liver diseases and also damage the liver. Primary injury is created by direct effects of Cd due to the binding of Cd^{2+} to sulfhydryl groups which are important molecules in mitochondria and result in oxidative stress. Cd directly destroys hepatocytes in the liver and causes hepatocellular injury. Secondary injury is produced by inflammation of several liver cells from acute Cd exposure (Rikans and Yamano, 2000).

Plant extracts are advised for the treatment of liver diseases due to the presence of antioxidants (Latha and Reddy, 2012). One hundred and seventy phytochemicals are isolated and have been reported to possess hepatoprotective activity and medicines have been used to treat various human diseases in ancient

and modern cultures (Narayanaswamy and Balakrishnan, 2011).

However, there is a need for less toxic, more effective, and cost-effective phytochemicals that act as antioxidants to scavenge free radicals and protect the body from the harmful effects of oxidative stress. Plants are used as food and source of medicines which possess secondary metabolites like phenolic compounds and flavonoids that are free radical scavengers. These metabolites are found in all parts of plants such as leaves, seeds, roots, fruits, and bark (Mathew and Abraham, 2006).

However, the dangerous side effects of synthetic drugs have encouraged scientists to move toward more natural medicines and the research in this area is growing fast (Thetsrimuang et al., 2011).

Extracts of plants are used for the cure of liver diseases such as hepatitis, cirrhosis, and loss of appetite and the *Terminalia arjuna* is a plant that is used for liver diseases (Lokendra et al., 2009). Herbal extracts are widely used for the treatment of liver diseases. Polyphenols and vitamins (C and E) are the major compounds of plants that act as antioxidants. It has been reported that the antioxidant properties of medicinal plants are due to the presence of phenolic compounds like phenolic acids, tannins, flavonoids, and anthocyanins (Djeridane et al., 2006).

Opuntia dillenii (Ker-Gawl) belongs to the family Cactaceae, commonly known as prickly pear or pear bush. It rapidly absorbs and stores water from the occasional rains of the dry regions. The plant has a thick cuticle which lowers the rate of transpiration. Leaves are in the form of spines; the stem has flat, leaf-like structures and performs the function of the leaf having water-storage tissues inside them. Juice of the fruit is purple in color and contains many rounded seeds (Allegra et al., 2007). *Opuntia dillenii* plant contains an excess amount of nutrients, such as phenolic compounds, polysaccharides, minerals, betalains, organic acids, lipids, vitamins, and amino acids. Traditionally *Opuntia* species are used as a source of medicines for the cure of diseases like gonorrhea, gastrointestinal problems, and inflammatory lesions. The flowers of the plant are used to treat respiratory diseases such as asthma and bronchitis. The succulent pads of *Opuntia* are a source of water for domestic animals in desiccated regions around the world and serve as a source of fodder (Gabremariam et al., 2006). They are also a wealthy source of natural antioxidants as a result of their flavonoid, ascorbic acid, and carotenoid contents. Betalain pigments found in cactus pears have sound effects on cell growth and inflammation (Siriwardhana et al., 2006). Betacyanin and betaxanthin are betalian derivatives that give red purple and yellow orange colour to cactus pear, respectively. These are water-soluble pigments that have strange antioxidant activities with harmless effects in humans (Livrea and Tesoriere, 2009).

Heavy metals are mixed in fertilizers absorbed by the plants, become part of the food chain, bioaccumulate in the bodies of organisms, and become toxic at a certain level (Praveena et al., 2013). The toxic level of heavy metals becomes carcinogenic to health and causes serious diseases such as gastrointestinal problems, lung, liver, and kidney and harm to the nervous system (Pandey et al., 2014). Heavy metals enter the body by inhalation in contaminated air, drinking water, and intake of

contaminated food (Gohil and Mankodi, 2013).

Justification of Research:

Environmental pollution is a universal problem, especially near the vicinity of the industrial zone. The fruit extracts have ameliorative potential against such noxious agents. Such type of study should be sponsored and scientists must reveal the slow poisoning materials that remain unnoticeable and common valuable fruits that are neglected should be added to food products to chelate the oxidative stress-producing agents.

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, Pakistan. They were kept in cages of 12" X 18" steel frame cages covered with wire gauze of small pore size. Paper cutting was used for bedding. The temperature was maintained throughout the experiment at 23 ± 2°C. Mice were supplied with a standard diet enriched with vitamins and dried milk. Drinking water was provided in glass bottles placed inverted and covered with cork-containing glass tubing for easy water gulping.

For the breeding purpose, one male mouse and two females were kept in one cage. As females became pregnant they were observed carefully. After pregnancy females were set aside in separate cages where they gave birth to young ones. Weaning was done one month before the young one became sexually mature. Required animals were used in experiments but some were left for further breeding purposes.

3.2 Dose groups

Thirty mice of (7-8) weeks of age and 30±1g were selected for the experiment and divided into three groups. Ten animals were used in each group.

1. **Control (untreated):** These animals were given a normal diet and regular drinking water.
2. **Cd treated group:** These animals were given 50ppm Cd ions in drinking water for 15 days along with normal food followed by Cd-free water for the next 7 days.
3. **Cd +OD group:** These animals were given Cd treatment as in the Cd group for 15 days while 0.2ml/12h of *O.dillenii* fruit extract was given to them in the next 7 days.

3.3 Preparation of Cd and O.dillenii fruit extract dose

Laboratory grade $CdCl_2$ was taken from the Chemistry lab of the Department of Chemistry, University of Sargodha; 1000ppm Cd ions stock solution was prepared by dissolving 1.79g of $CdCl_2$ in 1 Liter of water and 50ppm was then prepared by diluting 5ml stock solution with 950ml of water. Fruits of *O. dillenii* were collected from the area of Pai Khel in district Mianwali during December. Spines were removed from the fruit carefully and juice was obtained by pressing the fruit. The crude extract was then centrifuged at 500 rpm for 15 minutes and the purple supernatant was separated. For experimental use, this extract was stored at -30^{0}C as freshly thawed extract was used for animal treatment.

On the 23rd of the experiment, all animals were dissected for surgical removal of the liver. Organs were

weighted just after the dissection and were fixed in Cornoy's fixative for histological processing stained H&E and mounted in Canada balsam. Photomicrographs of the selected histological sections of the liver of experimental groups were obtained using Sony (DSC-W35) 7.2-megapixel digital cameras mechanically fitted on a Labomid CXR2 trinocular microscope, at 100×, 400× and 1000× magnifications. Photographs were processed in Corel DRAW 11 for the adjustment of color, sharpness, contrast, and digital cropping. The micrometric data was recorded from the digital photomicrographs of the histological sections of the liver with the help of a computer-assisted technique developed in our lab using Corel DRAW11. Ten of each animal randomly selected section from each group were used for the measurements of CSA of hepatocytes, their nuclei, and central hepatic lobular veins. The calibrations for each working magnification were made separately with the help of digital photo-shots of the stage micrometer on the same magnifications. To obtain various CSAs diameters were obtained with the help of right-angle perpendicular lines drawn across images of cells passing through the center. Data based on the micrometry was analyzed through SPSS software for ANOVA and Duncan's Multiple Range Test.

Results

The histological slides in the control group revealed all the signs of healthy liver microanatomy include the distribution of the hepatic layout in the form of lobules, with central veins. From where the hepatic cord radiates to the margins of each lobule. The hepatic cords were separated with hepatic sinusoids of appropriate size. Bi-nucleated hepatocytes frequently occur in the hepatic cords. On both sides, each hepatic cord was found to be laminated with unicellular layers of very thin kupffer cells (Fig 1 A). In the Cd-treated group, the hepatic cord has been found misaligned obliterating the intervening sinusoids almost completely. The nuclear size of the hepatocytes was large as compared to control. The cytoplasm appears vacuolated, and bi-nucleated hepatocytes were rarely visible. The kupffer cells were not identifiable, and apoptotic signs in terms of cellular debris were also visible (Fig 1 B). In the Cd+OFE group, the destruction of hepatocytes in hepatic lobules shows an intermediate pattern of above discussed two groups (Fig 1 C). Like control, there were present uni-nucleated and bi-nucleated hepatocytes with centrally placed rounded ovoid nuclei and cytoplasm not showing any signs of vacuolation. Like Cd group, many hepatocyte showed the above signs of enlarged nuclei with cytoplasmic vacuolation i.e. progressing toward apoptosis, and certain localized areas were also seen with the debris of apoptosis. The most interesting finding in this group was localized clumps of small undifferentiated hepatoblasts progenerator cells diffused through the scattered hepatocytes. The presence of hepatoblast in this group surely indicates the signs of hepatic regeneration.

Tab: 1 Ameliorative Role of OFE against Cd induced Hepatic anomalies

PARA METERS	GROUPS		
	C	Cd	Cd+ OFE
CSA hepatocytes μ^2	‡ 400.9±20.01 [a]	184.10±11.77 [b]	389.51±26.74 [a]

CSA hepatocytes nuclei μ^2	65.86±7.1 [a]	82.64±9.07 [b]	76.14±6.06 [c]
CSA hepatic vein μ^2	3280.20±1.83 [a]	6368.34±5.87 [b]	3824.64±2.17 [a]
Hepatocytes/6400 μ^2	11.60±.37 [a]	8.56±.32 [b]	10.84±.43 [a]
Regenerative (Oval) cell /6400 μ^2	2.32±.21 [a]	4.43±.23 [b]	6.76±.26 [c]

C; control, Cd; Cd treated, Cd+OFE; Cd and OFE post-treated group. Any two columns in the group not sharing a common lowercase alphabet vary significantly ($P \leq 0.05$), n=10, μ= micrometer, ‡; mean value.

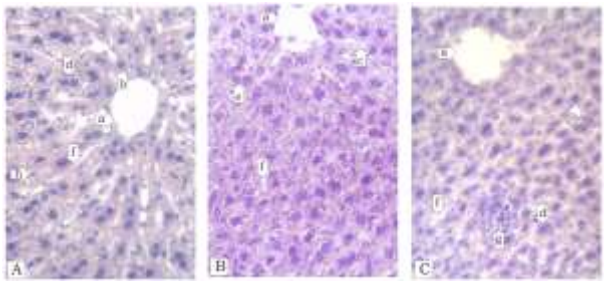

Fig 1: Selected section of liver from A: Control, B: Cd, C: Cd+OD groups.

[a: Central hepatic vein, b: Mononucleated Hepatocyte, c: Apoptotic cell debris, d: binucleated hepatocyte, e: Mega binucleated hepatocyte, f: Sinusoids, g: Regenrating hepatoblast, h: Kupffer cells]

Mean CSA of hepatocytes was recorded highest in C (400.9μ^2), followed by Cd+OFE (389.51μ^2) and Cd (184.10μ^2), and CSA of hepatocytes nuclei were recorded in Cd (82.64μ^2), followed by the Cd+OFE (76.14μ^2), and Control (65.86μ^2) while CSA of the central hepatic vein was recorded in Cd (6368.34μ^2), followed by Cd+OFE (3824.64μ^2) and C (3280.20μ^2) in descending order. The number of hepatocytes from highest to lowest in descending orders was recorded in C (11.60) > Cd+OFE (10.84) > Cd (8.56) and several regenerative (oval) cells per unit area were Cd+OFE (6.76) > Cd (4.43) > C (2.32). Data analysis (ANOVA) shows highly significant variation among the groups ($P \leq 0.0001$) and post hoc analysis indicates significant variation ($P \leq 0.05$) of the Control group from the other two groups.

Discussion

Liver is the main organ of metabolism and regulation of homeostasis. It has a key role in the detoxification of harmful chemicals (Ahsan et al., 2009). Many environmental toxicants cause various pathological changes in hepatic architecture (Robins et al., 2007). Cd is an environmental toxicant that has an injurious effect on the liver and damages the normal functioning of this vital organ (Stohs et al., 2001). In the present study injurious effect of Cd toxicity is observed in the liver of mice. Our results showed various histological changes in Cd affected liver. Central hepatic vein CSA was significantly increased. Sinusoids were messed up and not clear as a control group. Hepatocytes were decreased in number due to the Cd effect and their shape was irregular. Apoptotic hepatocytes were seen as cell debris. It has been reported that oxidative stress due to Cd causes lipid peroxidation that damages the cell membranes of the hepatocytes (Risso-de Faverney et al., 2004). Thus in the present study, the deceased hepatocytes might be due to membrane damage after Cd exposure. In our results, nuclear size was enlarged in the Cd-treated group as

compared to the control and Cd+OFE group. It has also been reported that Cd forms DNA adducts by binding with DNA strands, which affect DNA repair and nuclear size damage (Filipic and Hei, 2004). Several events may be involved in this process of DNA breakage. Studies revealed that ROS production due to carcinogenic metals is responsible for DNA injuries (Pourahmad et al., 2003). DNA is the target of toxicants and DNA damage is considered as the start of apoptosis (Chandra et al., 2000). The apoptotic hepatocytes in the current study were probably due to the interference of Cd to the DNA contents of hepatocytes.

In the Cd+OFE group signs of recovery were seen in the liver sections of animals reflecting the protective effect of *Opuntia* fruit extract. Hepatocyte shape was regular as compared to the Cd group. The size of the central hepatic vein decreased back to normal. Hepatocyte nuclei were rounded as of the control group. Regenerating heptoblast appeared in these sections which are a result of *Opuntia* fruit extract activity. A previous study suggested that liver damage was due to lipid peroxidation. However, phytochemical, antioxidants, and polyphenols present in plant fruit extracts can protect the liver from harm of toxicants. *Opuntia* fruit extract has phytochemicals which are strong antioxidants (Hatem et al., 2010) and protect the liver from the damaging effect of oxidative stress (Chang et al., (2008).

Phytochemicals like phenolic compounds like flavonoids (quercetin, kaempferol, and isorhamnetin) have the potential to prevent lipid peroxidation (Vijayan et al., 2003). Phenolic compounds are highly strong antioxidants and delay the prooxidative effect of proteins, lipids, and DNA by generating stable radicals (Tesoriere et al., 2005).

Recommendations

These hepatoblasts are precursors of normal hepatocytes and in our results, the signs of conspicuous hepatoblast regeneration indicate *Opuntia* fruit has hepatoprotective potential and recommended that fruit play an important role in antioxidant activity and lipid peroxidation against environmental pollutants especially Cd near the vicinity of big cities.

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8. Conclusion

Based on the results it was concluded that *O.dillenii* fruit extract possesses hepatoprotective and hepatolobular regenerative potentials. Thus, it should be further investigated for possible medicinal alternatives in various infectious and toxicological injuries to the liver in humans.

9. References

Ahsan, M.R., K.M. Islam and I.J. Bulbul. 2009. Hepatoprotective activity of methanol extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats. *Europ. J. Sci. Res*., 37(2): 302-310.

Allegra, M., L. Tesoriere and A.M. Livrea. 2007. Betanin inhibits the myeloperoxidase/nitrite-induced oxidation of human low-density lipoproteins. *Free. Radical. Res*., 41: 335-341.

Arisawa, K., H. Uemura, M. Hiyoshi, S. Dakeshita, A. Kitayama, H. Saito and M.

Soda. 2007. Cause-specific mortality and cancer incidence rates in relation to urinary beta2-microglobulin: 23-year follow-up study in a cadmium-polluted area. *Chem. Biol. Intrac*., 65: 155-164.

Chandra, J., A. SamalI and S. Orrenius. 2000. Triggering and modulation of apoptosis by oxidative stress. *Free Radic. Biol. Med*., 29: 323-333.

Chang, S.F., C.L. Hsieh and G.C. Yen. 2008. The protective effect of *Opuntia dillenii* (Haw). fruit against low-density lipoprotein peroxidation and its active compounds. *Food Chem*., 106: 569-575.

Dey, S.K., J. Dey, S. Patra and D. Pothal. 2007. Changes in the antioxidative enzyme activities and lipid peroxidation in wheat seedlings exposed to cadmium and lead stress. *Braz. J. Plant. Physiol*., 19: 53-60.

Djeridane, A., M. Yousfi, B. Nadjemi, D. Boutassouna, P. Stocker and N. Vidal. 2006. "Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds," *Food Chem*., 97: 654-660.

Filipic, M. and T.K. Hei. 2004. Mutagenicity of cadmium in mammalian cells: implication of oxidative DNA damage. *Mutat. Res*., 546: 81-91.

Gabremariam, T., S. Melaku and A. Yami. 2006. Effect of different levels of Cactus (*Opuntia ficus indica)* inclusion on feed intake, digestibility, and body weight gain in tef (*Eragrostis tef*) straw based feeding of sheep. *Anim. Feed Technol*., 131: 43-52.

Gohil, M.N. and P.C. Mankodi. 2013. Diversity of Fish Fauna from Downstream Zone of River Mahisagar, Gujarat State, India, *Res. J. Animal. Veter. Fishery. Sci*., 1(3): 14- 15.

Gupta, V. 2013. Mammalian Feces as Bio-Indicator of Heavy Metal Contamination in Bikaner Zoological Garden, Rajasthan, *Ind. Res. J. Animal. Veter. Fishery Sci*., 1(5): 10-15.

Hatem, S., M. Farghaly and A. Mohammed. 2010. Hussein. Protective Effect of Curcumin Against Paracetamol-induced Liver Damage. *Aust. J. Basic. Appl. Sci*., 4(9): 4266-4274.

Jarup, L. and A. Akesson. 2009. Current status of cadmium as an environmental health problem. *Toxicol. Appl. Pharm*., 238(3): 201-208.

Latha, L.S., and P.N. Reddy. 2012. Protective role of *Sphaeranthus amaranthoides* on hepatic marker enzymes. Glob. *J. Sci. Front. Res. Chem*., 12(2):47-50.

Liu, J., H.L. Huang, W.C. Zhang, and H. Li. 2010. Cadmium-induced increase in uterine wet weight and its mechanism. Birth Defects, *Res. B. Dev. Reprod., Toxicol*., 89: 43-49.

Livrea, M.A. and L. Tesoriere. 2009. Antioxidative effects of cactus pear [*Opuntia ficus-indica* (L) Mill] fruits from Sicily and bioavailability of betalain components in healthy humans. *Acta Horticult*., 811: 197-204.

Lokendra, S., K. Nameet and K. Pankaj. 2009. Reactive oxygen species (ROS), oxidative damage, and antioxidative defense systems with emphasis on herbal antioxidants and human and cattle health. *Biochem. Cell. Arch*., 9: 135-144.

Lushchak, V.I. 2011 Adaptive response to oxidative stress: Bacteria, fungi, plants and animals. *Comparative Biochemistry and Physiology*., 153: 175-190.

Mathew, S., and T.E. Abraham. 2006. In vitro antioxidant activity and scavenging effects of Cinnamomum verum leaf extract assayed by different methodologies. *Food Chem. Toxicol*., 44: 198-206.

Namjooyan, S., R. Khavari-Nejad, F. Bernard, S. Namdjoyan and H. Pir. 2012. The effect of cadmium on growth and

antioxidant responses in the safflower (*Carthamus tinctorius* L.) callus. *Turk. J. Agric*., 36: 145-152.

Narayanaswamy, N. and K.P. Balakrishnan. 2011. Evaluation of some Medicinal Plants for their Antioxidant Properties. *Inter. J. Pharm. Tech. Res*., 3: 381-385.

National Resources Canada. 2007. Canadian Minerals Yearbook.

Othman, M., H.S. Loh, C. Wiart, T.J. Khoo, K.H. Lim and K.N. Ting. 2011. Optimal methods for evaluating antimicrobial activities from plant extracts. *J. Microbiol. Methods*. 84(2): 161-166.

Pandey, G., S. Madhuri and A.B. Shrivastav. 2014. Fish Cancer by Environmental Pollutants, 1st Edn., Narendra Publishing House, Delhi, India.

Patra, R.C., A.K. Rautray and D. Swarup. 2011. Oxidative stress in lead and cadmium toxicity and its amelioration.*Vet. Med. Int*., 20: 45-73.

Pourahmad, J., P.J. Brien and F. Jokar. 2003. Carcinogenic metal induced sites of reactive oxygen species formation in hepatocytes. *Toxicol*., 17: 803-810.

Praveena, M., V. Sandeep, N. Kavitha and R.K. Jayantha. 2013. Impact of Tannery Effluent, Chromium on Hematological Parameters in a Fresh Water Fish, *Labeo Rohita* (Hamilton). *Res. J. Animal. Veter. Fishery Sci*., 1(6): 1-5.

Rikans, L.E. and T. Yamano. 2000. Mechanisms of cadmium-mediated acute hepatotoxicity.*J. Biochem. Mol. Toxicol*., 14(2): 110-117.

Risso-de Faverney, C., N. Orsini, G. Sousa and R. Rahmani. 2004. Cadmium-induced apoptosis through the mitochondrial pathway in rainbow trout hepatocytes: involvement of oxidative stress. *Aquat Tox*., 69: 247-258.

Robins, V., S.L. Kumar, and M. Fausto. 2007. The Liver, Gallbladder, and Biliary Tract. Basic *Pathology*., 8: 2-4.

Sheppard, S.C., C.A. Grant, M.L. Sheppard R.D. Jong and J. Long. 2009. Risk indicator for agricultural inputs of trace elements to Canadian soils. *J. Environ. Qual*., 38: 919-932.

Siriwardhana, N., F. Shahid and Y.J. Jeon. 2006. Potential antioxidative effects of cactus pear fruit(*Opuntia ficus-indica*) extract on radical scavenging and DNA damage reduction in human peripheral lymphocytes. *J. Food Lipid*., 13: 445-458.

Stohs, S.J., D. Bagchi, E. Hassoun, and M. Bagchi. 2001. Oxidative mechanisms in the toxicity of chromium and cadmium ions. *J. Environ. Pathol. Toxicol. Oncol*., 20: 77-88.

Tesoriere, L., D. Butera, M. Allegra, M. Fazzari and M.A Livrea. 2005. Distribution of betalain pigments in red blood cells after consumption of cactus pear fruits and increased resistance of the cells to ex vivo-induced oxidative hemolysis in humans. *J. Agr. Food Chem*., 53: 1266-1270.

Thetsrimuang, C., S. Khammuang, K. Chiablaem, C. Srisomsap and R. Sarnthima. 2011. Antioxidant properties and cytotoxicity of crude polysaccharides from *Lentinus polychrous Lev. Food. Chem*., 128: 634-639.

Vijayan, P., H.C. Prashanth, S.A. Dhanraj, S. Badami and B. Suresh. 2003. Hepatoprotective effect of total alkaloid fraction of *Solanum pseudocapsicum* leaves. *Pharmaceut Biol*., 41: 443-448.

Zhang, W.C., F. Pang, Y.Q. Huang, P. Yan and W. Lin. 2008. Cadmium exerts toxic effects on ovarian steroid hormone release in rats. *Toxicol. Lett*., 182: 18-23.