VOL. 9, NO. 5, MAY 2014 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science

www.arpnjournals.com

EFFECT OF HYDROLYZED LEATHER SHAVINGS AS FOOD ADDITIVES, PARTIALLY REPLACING VEGETABLE PROTEINS IN THE FISH FOOD, ON HISTOPATHOLOGY OF ROHU (*Labeo rohita*) fingerlings

Saeeda Fatima[1], Muhammad Anwer Malik[1], Ali S.[2] and Usman S.[2]
[1]Department of Zoology, GC University Lahore, Pakistan
[2]Food and Biotechnology Research Centre (FBRC), PCSIR, Ferozpur Road, Lahore, Pakistan
E-Mail: saeeda.uzair@yahoo.com

ABSTRACT

The effect of hydrolyzed chrome shavings on the histopathology of liver kidney, stomach and intestine in the fingerlings of *Labeo rohita* were investigated using 5%, 10% and 15% levels of chrome shavings. After supplementation histopathology of liver Kinney, stomach and intestine showed varying degrees of changes such as necrosis, pyknosis, karyolysis, hemorrhages and degeneration of epithelial linings in the tissues of groups exposed to chrome shaving. Results showed that these changes are time and dose dependent.

Keywords: *labeo rohita*, karyolysis, pyknosis, necrosis, chrome shavings, histopathology, degeneration of epithelial lining.

INTRODUCTION

The estimated human population of Pakistan at present is 150 million and is increasing at a rate of 20.17 per year making it obligatory to explore the ways for a rapid intensification of food resources. (Anonymous, 1996). Pakistan being a developing country is trying to improve the nutritive resources and commercial exploitation of fish can be economically beneficial and that can be used to overcome nutritive deficiencies. Fish could also be a useful biological model for studying teratogenic, carcinogenic and mutagenic effects of environmental chemicals. (Krishnaja and Rege, 1982). Due to considerable expense of fish meals and concern regarding their limited availability as aquaculture continuous to expand, evaluation of appropriate substitutes for fish meal has been a long standing research priority (Hardy; 1999) Various kinds of comprising variety of ingredients are being used as fish meal. But the use of material for fortifying meal is an alternative for cheap and better production of fish at local level coupled with a possibility of decreasing the possible pollution risk. (Cuzon *et al*., 2002).

Chrome shavings, which are obtained during leather processing, could be used, after hydrolyzing, as a food additive for the fish with a possibility of reducing pollution and getting an access to a cheaper food source. Chrome shavings, the by product of tannery industries is used in the diet of poultry in some developed and developing countries (Alam, *et al.*, 2002). Chrome shavings may be used in fertilizer production and making different feeds for live stock. Leather trimmings and shavings are used for leather meal production which contains certain metals, most notably Cr, Cd and Zn (Waters, 1989 and Parker, 1999). AAFCO allows the use of leather shavings as feed additive in non-ruminants. Prussian carp (*Caressius auratus* gibelio) investigated 1000m above and 400m below the discharge points of leather waste products, showed that the exposure of fish to various concentration levels (sub-lethal) of chromium caused an increase in the frequency of micronuclei (Al-Sabti *et al*., 1999) Rainbow trout (*Salmo gairdneri*) when exposed to Na_2CrO_4 solution of different concentrations (2-50 mg/l Cr at pH (7.8 and 6.5), the highest contents of Cr were found in gills, liver, kidney and digestive tract of the trout . Cr was concentrated in nuclear fractions of the gill tissue and in the soluble fractions of kidney and liver tissue. In gills Cr was more concentrated at pH 6.5 than at pH 7.8 irrespective of exposure time and concentration (Vander Puttel *et al*., 1981) Intrabdominal injection of chromium salts to the bony fish *Myxocephlus scorpius*, produced nephrotoxic effects which included the disturbance in the magnesium secretion. One day after the injection of chromium compound, total damage of the proximal tubules was observed (Gambarian and Larova., 1992). Rainbow trout (*Oncorhynchus mykiss*) when maintained on high chromium diet showed a positive influence on serum lysozyme activity. The respiratory burst of head kidney macrophages was also examined (Gatta *et al*., 1997).

Chromium altered the morphological development of vital tissues and organs in the bodies of young fish *Pondivis reticulate*. Liver regions were enlarged. Tissue regions in the liver developed scarlet lumps of blood and decayed tissue (Brown *et al*., 1998) increased efficiency of nutrient utilization is particularly important as various sectors of the aquaculture industry are projected to expand and intensify production in the decade to meet the ever increasing demand for sea food (Tidwell and Allam., 2002)

MATERIALS AND METHODS

In the present investigations fingerlings of a farmed fish *Labeo rohita* commonly called rohu was used as experimental animal. Three levels of chrome shavings (procured from local tannery industry at Kasur) 5%, 10% and 15% as 20% of fish body weight were prepared. Studies were carried out for 200 days. Histological studies were carried out by Drury and Wallington's method (1980).

Processing of tissues for histopathological studies

The tissue being removed from the fish was rinsed in 0.85% saline solution for three times to remove any blood or debris attached on external surface. The tissues were then cut into small pieces of approximately 3-5mm, keeping in view the orientation of the tissue.

Fixation of tissues

The aim of fixation is the preservation of cells and tissue constituents in a condition identical to that existing during life, prevention of autolysis, loss of easily diffusible substances by appropriate coagulation and fortification of the tissues against the deleterious effects of the various stages in tissue processing. In the present study the tissues were fixed in Bouine's fluid. The tissue was kept in fixative for 6-24hrs depending upon the size of the tissue (Drury and Wallington, 1980).

Processing of tissues

The preparatory treatment before being sectioned, entailing impregnation of the specimens with an embedding consistency for microtomy is tissue processing. In present study paraffin wax method was used for the processing of tissues, which is as follows:

Dehydration: It was carried out by immersion of tissues in following grades of alcohol:

- A number of washes with 70% alcohol to get rid of picric acid.
- One change with 90% alcohol for 16-24 hrs.
- Two or three changes with absolute alcohol for 5-6hrs. (Drury and Wallington, 1980).

Clearing: For clearing two washes of xylene were given to the tissues for 15mins each.

Wax impregnation: To remove clearing agent and completely permeate the tissue sby paraffin wax to produce a block from which sections may be cut, tissues were directly transferred from xylene to the molten paraffin wax (56-58C) for overnight in oven (Drury and Wallington, 1980).

Embedding: Ordinary cavity blocks of glassware were used as mould for block making. To lubricate a thin layer of glycerol was placed in cavity. Molten wax was poured and allowed to stand for sometimes. With warm forceps tissues were taken and put in the middle of cavity which was then placed in cold water and blocks were carefully removed (Orchin, 1967)

Trimming of wax block: Surplus wax was cut from the sides of the blocks and trimmed so as to leave a 2-3mm margin of wax.

Section cutting: Thickness of sections was selected between 8-19µm in the form of ribbons of 10-15cm in length. Then it was detached from the knife by inserting a scalpel blade.

Mounting: A mixture of egg albumin and glycerol was smeared very thinly on slide before mounting of section. Section was gently lowered onto the surface of the water (5-10C) below the melting point of the wax.

After removal of wrinkles sections were flattered by this gentle heat (Drury and Wallington, 1980).

De waxing: After slightly warming the slides on a gentle flame the de-waxing was carried out by giving two washes in xylene for 5mins each.

Hydration: Sections were run through downgrading of alcohol (one rinse each) for hydration which is as follows:

90%, 70%, 50%, 30% alcohol for 2-5mins.one rinse with distilled water for 2-5mins was also done.

Staining: For distinguishing purpose tissues are stained. They were stained with Haematoxylin for 15-20mins. Then washed the slides under running tap water for 2-3mins.excess stain was removed by quick dip of 0.5-1% HCl in 70% alcohol for a few seconds. Then stained the slide in 1% aqueous Eosin for 1-3mins. In the last slide was dipped into 90% and 100% alcohol for a few minutes (Drury and Wallington, 1980).

Oiling: For the transparency of section oiling was carried out by placing the slides into clove oil for 2-3mins.

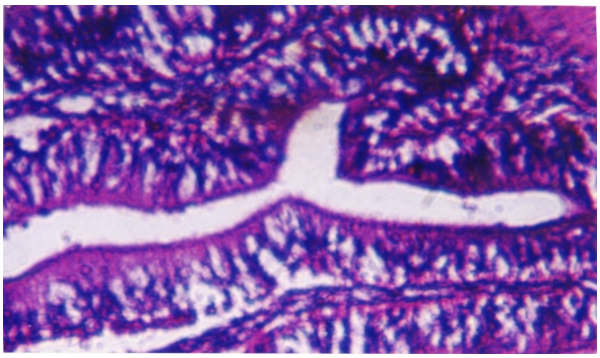
Mounting: To prevent contamination with dust and air mounting was done. Candabalsam was used on the slide and cover slip was placed. For drying and preservation kept the slides at room temp for 3-4days.

RESULTS AND DISCUSSIONS

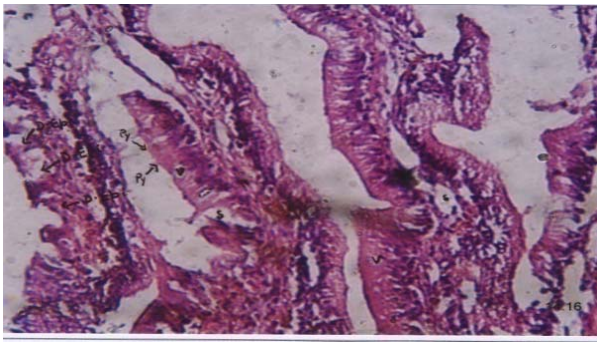
Histopathpological studies

Stomach

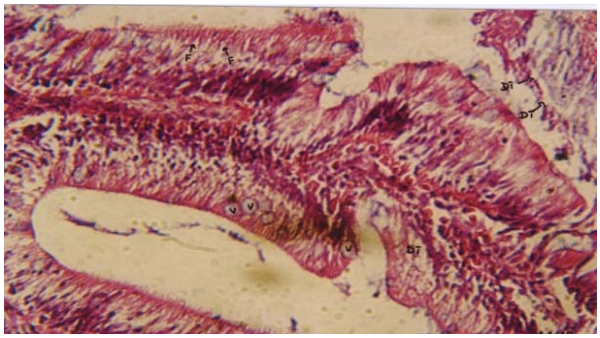
The exposure of fingerlings of fish *Labeo rohita* to the three levels of chrome shavings i.e., 5, 10 and 15% remarkably disturbed the normal histology of the stomach. The most common changes observed were pyknosis, necrosis, and disruption in the outer lining of longitudinal muscles, degeneration and development of fibrosis. These were also accompanied by the degeneration of epithelial lining. Moreover, gaps also appeared between the gastric glands and vacuolization was also apparent in the gastric glands epithelium. Our results were also comparable to that of Kaoud and *et al.* who treated *Oreochromis niloticus* with cadmium. The changes in histology of fish were vacuolization of sub-mucosal tissues, degeneration of serosal layer, columnar epithelium, goblet cells and basement membrane. Secretory cells were also distorted. Similarly Yashikawa *et al*. (1960) observed histological changes in stomach and intestine of rats after feeding Cadmium stearate (0.03% cadmium diet) for 90 days. All test animals also had growth suppression. Tarasub and *et al.* (2009) when treated rats with cadmium they observed damage to the gastric glands and a number of vacuoles in the cells of gastric glands epithelium.



T.S of control group of fingerlings of *Labeo rohita* showing normal histology at 400x.



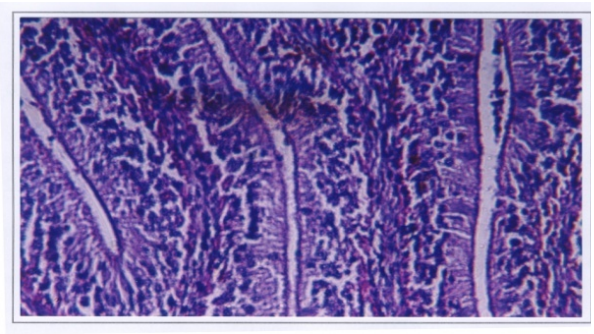
T.S of stomach of fingerlings of *Labeo rohita* showing degeneration of epithelial lining (D.Epi), pyknosis (Py), Spaced(S) and vacuolization (V) after exposure to 15% level of chrome shaving for 200days at 400x.



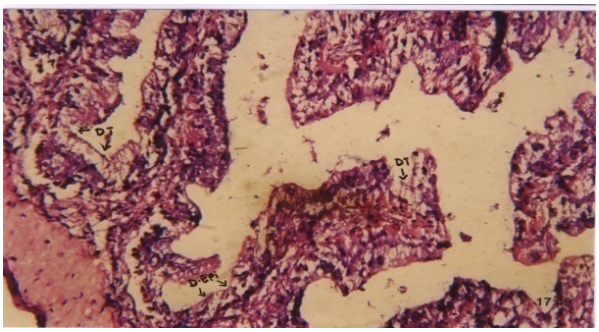
T.S of stomach of group B showing degeneration of tissues (DT), fibrosis (F), Vacuolization (V), degeneration of epithelium after exposure to 5% level of chrome shavings for 200 days at 400x.

Intestine

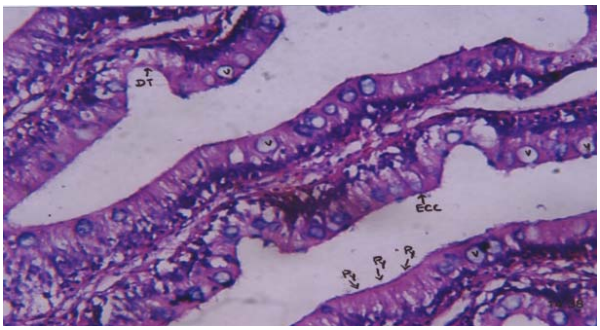
The intestine showed disruption in the lining of the villi (De. Epi) and pyknosis (Py). Degeneration of tissue (DT) and of the epithelial lining was obvious. The structure of the cryptic cells was highly degenerated. After prolonged treatment extensive degeneration of intestinal villi, vocuolization and fibrosis occurred. Elongation of cryptic cells (ECC) and infiltration (I) was also seen. The changes were time and dose dependent. Similar damages were observed by Mughal and Hashmi (2000) after exposing farmed fish *Ctenopharyngdon idella* (Grass carp) to sublethal contrations of potassium dichromate. Major histological changes were necrosis, pyknosis, degeneration of mucosal epithelium and extensive vacuolization. Our results were also comparable to that of Sastry and Gupta (1998) who exposed *Channa punctatus* to 6.8mg/l of lead nitrate and observed in intestine and pyloric caeca the flattening of villi, inflammation and necrosis. Kaoud and *et al.* (2011) had the same results after exposing *Oreochromis niloticus* to cadmium. Major histological changes were degeneration of columnar epithelium, distortation of basement membrane of the villi, necrosed mucosa and submucosal hemorrhage. Cadmium treated rats showed shorter and thicker villi with a large number of vacuoles and infiltration of neutrophils in the lamina porpia. Velmurugan and *et al*. (2007) when exposed *Cirrhinus mrigala* to sub lethal concentrations (0.3ppb and 0.6ppb) of lambda cyhalothrin (a synthetic pyrethroid pesticide) observed intestinal lesions, infiltration of eosinophils into lamina porpia and atrophy of epithelial cells. Flattening and cracked clay appearance was observed by Bhatnagar and *et al.* (2007) after chronic exposure for 30, 60, 90 and 120 days of fingerlings of *Labeo rohita* to 15mg NaF/L.



T.S of intestine of control group of fingerlings of Labeo rohita showing normal histology at 400x.



T.S of intestine of fingerlings of *Labeo rohita* showing degeneration of tissue (DT) and of epithelial lining (D.Epi) after exposure to 5% level of chrome shaving for 70days at 400x.

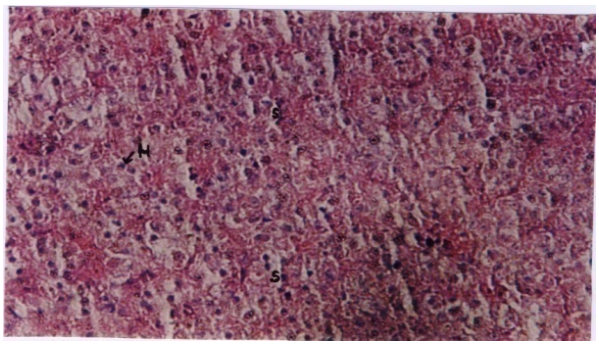


T.S of intestine of fingerlings of *Labeo rohita* showing degeneration of tissue (DT), elongation of cryptic cells (ECC), pyknosis (Py) and vacuolization (V) after exposure to 15% level of chrome shaving for 200days at 400x.

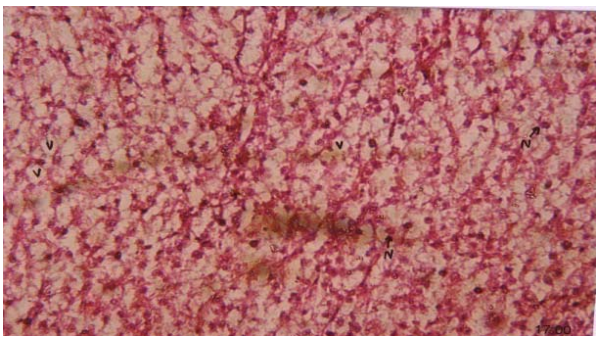
Liver

The histology of the liver showed extensive damage to the liver of the treated fish. The effect was time and dose dependent. The changes observed were gaps between hepatcytes, pyknosis (Py) necrosis (N) cirrhosis (C) and degeneration of tissue (DT). After prolonged treatment vacuolization and necrosis became more prominent in al the three treated groups. Results were comparable to that of Rajeshkumar and Munuswamy (2011) who treated the milk fish (*Chanos chanos)* with polluted water having heavy metals (Cu, Pb, Zn, Cd, Mn and Fe). Liver of treated fish showed rupture of the central vein, irregular hepatic plate, vacuolation and congestion of blood vessels in the hepatocytes. Mishra and Monhanty (2009) when exposed *Channa punctatus* to 2mg/l for 2 months caused shrinkage of hepatocytes, vacuolization at localized areas. Increase in pyknotic cells was also aobserved.4mg/l for two months caused marked increase in sinusoidal spaces. Similar changes were observed in our studies. Jafri and Sheikh (1999) also demonstrated liver necrosis and pyknosis in tilapia following chromium estimation. Brown *et al*., (1998) exposed the fish to contaminated water containing chromium and lead for a twelve-week period and observed the development of scarlet lump of blood and decayed tissue in tissue regions of young fish *Pondivis reticulata*. In the present studies similar changes were noted which seemed to be due to Cr accumulation. Similar studies were done by Khangarot and *et al*. (1999) exposing fresh water catfish *Saccobranchus fossilis* to subchronic levels of Cr, 1, 1.0 and 3.2 mg/liter Cr) and it was seen that Cr accumulation in liver, kidney and spleen was dose dependent.

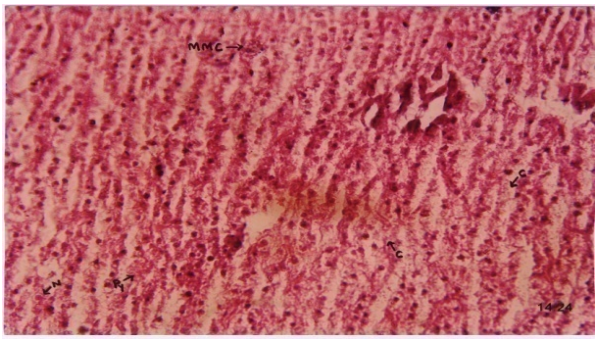
The predominant changes in our studies were pyknosis and necrosis. Similar results were obtained by Sastry and Gupta (1998) after exposing the teleost fish *Channa puctatus* for 125 days to sublethal concentration (6.8mg/1) of lead. The degenerative changes produced in the liver were damage in the form of liver, disarray, necrosis, inflammation of portal areas, hardening of connective tissue, shrinkage of nuclei, septa formation around blood vessels and lipofucsin granules accumulation in the cytoplasm of hepatocytes.



T.S of liver of fingerlings of *Labeo rohita* (control group) showing normal structure of hepatocytes (H) and Sinusoids (S) at 400x.



T.S of liver of fingerlings of *Labeo rohita* showing extensive necrosis (N) and Vacuolization (V) after exposure to 10% level of chrome shavings for 200 days at 400x.



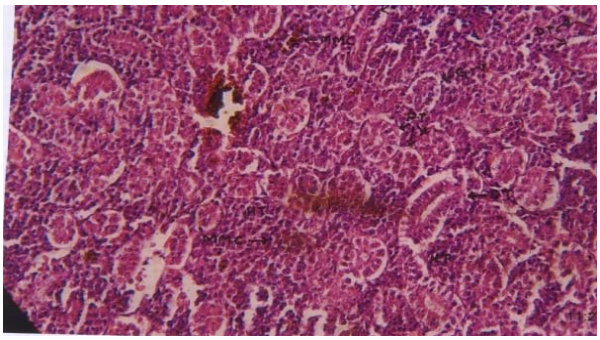
T.S of liver of fingerlings of *Labeo rohita* showing extensive cerrhosis(C), necrosis (N), pyknosis (Py) and melanomacrophage centre (MMC) after exposure to 15% level of chrome shavings for 200 days at 400x.

Kidney

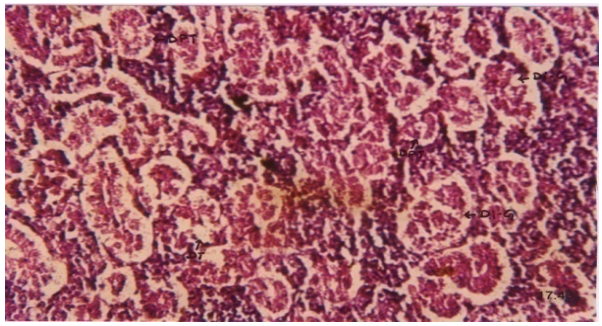
Kidneys of the treated fingerlings showed disorganization of the glomerulules, (Di.G), degeneration of the epithelial lining of the proximal and distal tubules (D.PT and D.DT). After prolonged treatment the structural profile was much disturbed that the various regions of the kidneys were not easily identifiable. As the dose and time period increased renal necrosis and glomerular degeneration also increased. Our results were comparable

to that of Wasfi and Adam (1976) who reported that intravenous administration of copper caused dilation and necrosis of kindney tubules in goats. Similarly Farag and *et al*. (2006) observed that Chinok Salmon (*Oncorhynchus tshwystscha*) had gross and microscopic lesions after treatment with both concentrations 24/120 and 54/266µg $Cr1^{-1}$ causing necrosis of cells lining the kidney tubules and dilation of tubules. Their lamina also contained scattered cellular debris, eosinophilic protein and tubular epithelial cells varied from necrotic to attenuate.

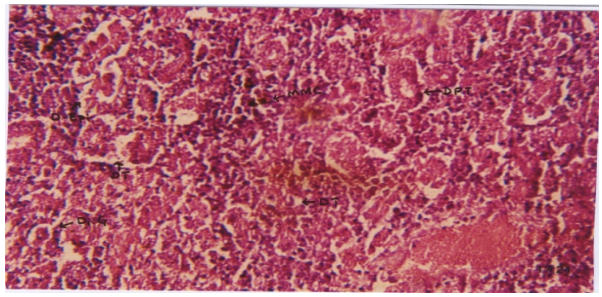
Riaz and Mughal (2000) reported dilation of epithelial cells in renal tubules and glomerulus necrosis pyknosis, degeneration and disintegration of haemopoietic tissues and tubules after exposing grass carp *Ctenopharyngodon idella* to different sub lethal levels of Cr, i.e., 60, 80, 100mg/l of $K_2Cr_2O_7$. Akram *et al*., (1999) reported shrinkage of glamomerular tuft, disintegration of proximal tubules and parenchymatous cells of haemopoietic tissues in the kidneys of *Baralius vagra* when exposed to cadmium and zinc. Dubale and Shah (1980) also reported disintegration and vacuolization of glomruli of *Channa punctatus* after exposing to cadmium. The *Labeo rohita* showed when exposed to chromium that initially there was disorganization which later resulted in the disintegration of glomerular tuft. In this respect our results agree with those of Dubale and Shah (1980). Similar changes were also observed in all treated groups in our studies. But in all the three treated groups after 200 days high degree of degeneration in proximal and distal tubules was observed and it appears that this extensive damage occurring in all the organs studied was probably due to high dose levels (5%, 10% and 15%) and also the prolonged exposure to the chrome shavings. Similarly when Mishra and Monhanty (2009) when exposed *Channa punctatus* to 2mg/l of Chromium caused contraction of glomerulus and increase of spaces inside Bowman's capsule. At 4mg/l for two months lesions in renal tubules and pyknosis of epithelial cells were prominent changes in histology of kidney. The intensity of changes was less at 2mg/l and changes more prominent at 4mg/l.



T.S of Kidney (control group) of fingerlings of *Labeo rohita* showing normal arrangement of distal tubules(DT), glomerlus (G), haemopoietic tissue, melanomacrophage centre (MMC), and proximal tubules (PT) at 400x.



T.S of kidney of fingerlings of *Labeo rohita* (group C) treated with 10% level of chrome shavings for 70 days showing extensive disorganization of glomerulus (Di.G), degeneration of tissue (DT) and of proximal tubules (Di.P).



T.S of kidney (group C) of fingerlings of *Labeo rohita* treated with 10 % level of chrome shavings for 200 days showing degeneration of tissue (DT), degeneration of proximal tubules (DPT), degeneration of epithelial lining (D.Epi), disorganization of glomerulus (Di.G), infiltration and melanomacrophage cemtre (MMC) at 400x.

CONCLUSIONS

This study shows that *Labeo rohita* fingerlings when exposed to three levels of chrome shavings i.e., 5, 10 and 15 %, show various degrees of degenerative changes in histopathology of stomach, intestine, liver and kidney. These changes are not affecting too much when at low levels (5%) but become extensive with increasing dose and time period. So chrome shavings could be used for shorter time duration but feeding of this food additive for a longer period of time is not recommended.

REFERENCES

Al-Sabti K., Franko M., Andrijania C.B., Knez S. and Stegnar P. 1999. Chromium induced micronuclei in fish. Journal of Applied Toxicolology. 14(5): 333-6.

Anonymous. 1996. Economic Survey 1995-96 Govt. Pak, Finance Division, Islamabad, Pakistan.

Akram M., Hafeez M. and Nabi G. 1999. Histopathological changes in the kidney of a fresh water cyprinid fish, *Barilius vagra*, following exposure to cadmium. Pak. J. Zoo. 31: 77-80.

Alam M. A., Amin M. R., Samand M. A., Islam M. A. and Wadud M. A. 2002. Use of tannery waste in the diet of broiler. Asian-Aust. J. Anim, Sci. 15(12): 1773-1775.

Bhatnagar C., Bhatnagar M. and Regar B.C. 2007. Fluoride-Induced Histopathological changes in Gill, Kidney and Intestine of Fresh water Teleost *Labeo rohita* Res. Rep. Fluoride. 40(1): 55-61.

Brown L. R., Booker T. M. and Miller R. H. 1998. Environmental contamination effects of inorganic lead and chromium: teratogenic effects on the liver, gills and stomach of *Pondivis reticulata*, *Teratology.* 57(415): 242.

Cuzon G., Gaxiola T., Sanchiz A. and Aquacop. 2002. Raw ingredients for marine aquaculture fresh. Infremer COP BP 7004 Taravao, Tahiti, French Polynesia. UNAM, Ciedad dal Carmen, Campeche, Mexico. Universi dad de La Havana, Cuba.

Drury R. A. B. and Wallington E.A. 1980. Carleton's Histological Technique. Oxford University Press, United Kingdom. 127: 44-55.

Dubale M. S. and Shah P. 1981. Biochemical alterations induced by cadmium in the liver of *Channa punctatus*. Environ. Res. 26(1): 110-118.

Farag A. M., May T., Marty G. D., Easton M., Harper D. D., Little E. E. and Cleveland L. 2006. The effect of chronic chromium exposure on the health of Chinook salmon (*Oncorhynchus tshawytscha*), Aqua. Toxicol.76: 246-257.

Gambarian S. P. and Lavrova E. A. 1992. Nephrotoxic action of Platinum, Chromium and Cadmium Compounds on marine bony fishes. Mutat. Res. 102(1): 71-82.

Gatta J. P., Rutland F. H. and Buljan. 1997. Pollution limits for discharge of tannery effluents into water bodies and seaers world leather. 9: 65-68.

Hardy R. H. 1999. Aquaculture's rapid growth requirements for alternate protein sources. Feed management. 50: 25-28.

Mughal M. S. and Hashmi S. 2000. Chromium induced changes in gastrointestinal tract of grass carp (*Ctenopharynogodon idella*). Biologia. 46: 69-74.

Jafri S. I. H., Sheikh S. A. and Dewan V. K. 1999. Toxicity and bioaccumulation of chromium in tilapia, *Oreochromis mossambicus.* Pak. J. Zoo. 3141: 106-108.

Kaoud H. A., Zaki M. M, E I Dashan A. R., Saeid S and E. I. and Zorba H. Y. 2011. Amelioration the Toxic Effects of Cadmium-Exposure in Nile Tilapia (*Oreochromis niloticus*) by using *Lemna gilba* L. Life Sci. 8(1): 185-195.

Khangarot B. S., Rathore R. S. and Tripathi D. M. 1999. Effects of chromium on humoral and cell-mediated immune responses and best resistance to disease in a fresh water catfish, *Saccobranchus fossitis* (Bloch). Ecotoxicol. Environ. Saf. 43(1): 11-20.

Krishnaja A. P. and Rege M. S. 1982. Induction of chromosomal aberrations in fish *Boleophthalmus dissumieri* after exposure in vivo to mitocycin C and heavy metals mercury, selenium and chromium. Mutat Re. 102(1): 71-82.

Mishra A. K. and Monhanty B. 2009. Chronic exposure to sublethal hexavalent chromium effects organ histopathology and serum cortisol profile of a teleost, *Channa punctatus* (Bloch). Sci. Total Environ. 40(7): 5031-5038.

Parker W. R. 1999. Arsenic in the marine and aquatic environments: Analysis, Occurrence and Significance. Crit. Rev. Environ. Con. pp. 465-482.

Rajeshkumar S. and Munuswamy N. 2011. Impact of metals on histopathology an d expression of HSP 70 in different tissues of milk fish (*Chanos chanos*) of Kattuppalli Island, South East Cost India. Chemosphere. 83: 415-421.

Riaz S. and Mughal M. S. 2000. Histopathological changes in kidney of grass carp, *Ctenopharyngodon idella* as manifested by chromium intoxication. Biologia. 46: 83-88.

Sastry K. V. and Gupta P. K. 1998. Histopathological and enzymological studies on the effects of chromic lead nitrate intoxication in the digestive system of fresh water teleost, *Channa punctatus.* Environ. Res. 17: 472-479.

Tarasub N., Tarasub C. and Ayuttaya W. D. N. 2009. Histological changes of Spleen, Stomach and Small Intestine Induced by Cadmium in Rats and the Protective Effect of Curcumin. Thamm. Med. J. 8(3): 213-224.

Tidwell J. H. and Allah G. L. 2002. Fish as food: aquacultures contribution. World Aqua. 33: 44-42.

Van der Puttel M. B., Faber, R., Zamora J. E. and Verdegem M. C. J. 1981. Effect of feeding level on feed losses and feed utilization of soya and fish meal diets in *Colossoma macropomum* (Cuvier). Aqua. Res. 28: 391-403.

Velmurugan B., Selvanayagam M., Cengiz E. I. and Unlu E. 2007. Histopathology of lambda-cyhalothrin on tissues (Gill, Kidney, liver and intestine) of *Cirrhinus mrigala*. Environ. Toxicol. Pharm. 24: 286-291.

Wasfi I. A. and Adam S. E. I. 1976. Histopathological and haematological changes induced by Copper in sheep. J. Comp. Path. 86: 387-391.

Waters J. B. 1989. In: General Pathology, 6th EdChurchill Living Stone London. . pp. 470-479.

Yashikawa H., Hara N. and Kawai K. 1960. Experimental studies on Cadmium stearate poisoning Dissociation and Toxicity. Bull. Nah. Inst. Ind. Health.