INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

HEPATOTOXIC AND NEPHROTOXIC EFFECTS OF CHRONIC LOW DOSE EXPOSURE TO A MIXTURE OF HEAVY METALS – LEAD, CADMIUM AND ARSENIC

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ABSTRACT

Objective: Cadmium (Cd), lead (Pb) and arsenic (As) are known to cause adverse health effects on various human and animal systems. In the present study, we evaluated effects of chronic low dose exposure to a mixture of Pb, Cd and As on liver and kidney of male rats. The findings were compared with the finding on individual exposure to each of these metals at the dose at which it was present in mixture. **Methods:** The animals were treated with the mixture of Cd, Pb and As, and with each individual metal at a very low dose for 90 consecutive days. Following the treatment, animals were autopsied and serum glucose, albumin, total protein, total cholesterol, urea, creatinine, glutamicoxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT) were estimated. The enzymes alkaline phosphatase (ALP) and acid phosphatase (ACP) were measured from liver and kidney tissue homogenates. The histopathological changes in liver and kidney were evaluated. **Results:** There was a significant increase in serum glucose, cholesterol, urea and creatinine in mixture treated rats. The serum protein and albumin levels were decreased in mixture treated animals. There was also a significant increase in the activity of serum enzymes, SGOT and SGPT. The activity of enzymes ACP and ALP from both liver and kidney were increased. Histopathological changes in liver and kidney tissues exhibited toxic symptoms in treated animals. Low dose exposure to individual metal component used in the mixture did not show much difference in study parameters in comparison to the control animals. **Conclusion:** Chronic exposure to a mixture of heavy metals cadmium (Cd), lead (Pb) and arsenic (As) at a very low environmentally relevant dose produced hepatotoxic and nephrotoxic effects in albino rats.

Keywords: Mixture of heavy metals, chronic low dose, hepatotoxicity, nephrotoxicity.

INTRODUCTION

Heavy metals are found naturally in earth and become concentrated as a result of human and animal activities. Heavy metals enter plant, animal and human tissues via airinhalation, diet and manual handling. Metal pollution has harmful effect on biological systems and does not undergo biodegradation[1]. Most of our knowledge concerning the health effects of toxic metalslargely stems from studies conducted on populations with relatively high exposure usually to individual metal in industry or in heavily polluted environments.People are usually exposed to a combination of metals in their living and working environment. Very few studies have addressed the possible effects of chronic low environmental exposure to mixture of these metals. The toxic effects of mixture of heavy metals, especially at low, chronic environmentally relevant doses are poorly recognised[2]. Metals can interact in human body as soon as they are absorbed, when they bind with specific proteins, or during transport and distribution in the body. Their interaction can either increase or decrease the toxic action of single components[3]. Reports on the effect of

long term combined exposure to environmental pollutants are scarce. Furthermore, their effects, especially those of heavy metals, are usually tested on the basis of acute or sub-chronic exposures that do not reflect the actual risk to general population[4].

Lead is one of the heavy metals that occursnaturally in the environment, but its industrial use has resulted in increased levels of the metal in soil, water and air. Lead toxicity occurs from low level exposures from various environmental sources including air, food and water. Leaded petroleum is one of the major sources of lead pollution[5]. Cadmium, another heavy metal found as non-essential trace element present as contaminant in food, water and tobacco leaves, it can either be ingested is a contaminated food or inhaled[6]. General population, especially in industrial areas are exposed to low dose of lead (Pb) and cadmium (Cd). Dietary and other forms of intake of both the metals are nearing the tolerable limits of these metals in various cities and various countries[7]. Chronic exposure to these metals is associated with many diseases such as liver disorders, cancer, neurological disease and osteoporosis[8]. It is also associated with elevated blood pressure and contributes to the development of cardiovascular disease[9].On the other hand, arsenic is a common environmental toxicant and a naturally occurring metalloid available in earth crust and biosphere[10]. Oral and inhalation are the main routes by which humans are exposed to arsenic. Ground water contamination of arsenic is one of the major problems for India and adjoining countries[11]. The other sources of arsenic contamination of environment include the use of pesticides in agricultural fields, mining and preservation of timber[12].

Among possible target organs of heavy metals liver, kidney and neural systems appear to be the most sensitive one[13].Chronic exposure to Pb showed nephropathy, including nephromegaly and dysfunction of proximal tubules in animal studies[14]. The lead is conjugated in liver after absorption and pass to the kidney. A minimum quantity of lead is excreted in the urine and the rest of it is stored in different organs of the body and ultimately affects many biological activities at molecular, intercellular and cellular levels. As a result different morphological alterations occur even after levels of lead fall down [15]. Cadmium is transported in the plasma after absorption and binds to albumin. It accumulates mainly in the kidney and liver. Cadmium causes reproductive disorders, renal and hepatic dysfunction, osteomalacia, neurological impairment and changes in pancreatic activity[16]. Chronic exposure to arsenic is reported to cause diabetes mellitus, hypertension, and asthma, cancers of skin, liver, kidney, bladder and prostate andis also reported to be fetotoxic and teratogenic[17].

All most all the toxicological studies on these water contaminating heavy metals deal with the single metal exposure at a dose much higher than World Health Organisation (WHO) suggested maximum permissible limit (MPL) of a single metal in drinking water. Little is known about the interaction among these heavy metals due to their co- exposure through drinking water and their combined toxic effects on plants, animals and humans. Therefore the aim of our study was to address interaction of metals at environmental doses by assessing the effects of long-term exposure to combination of Cd, Pb and As at low doses on liver and kidney in rats.

MATERIAL METHODS

Chemicals and Drugs

The Cadmium chloride ($CdCl_2$) and Lead (II) acetate anhydrous were obtained from the Hi Media Laboratory Pvt.Ltd.India. Sodiumarsenate was obtained from Nice Chemical Pvt Ltd. India. The Harris haematoxylin solution was obtained from the Merck Pvt. Ltd. Eosin yellow staining solution was obtained from Central Drug House (P) Ltd. The Commercial kits such as glucose kit, total protein kit, albumin kit, cholesterol kit, urea kit, creatinine kit, SGPT kit, SGOT Kit were obtained from the Coral System. Magnesium chlorides, sodium hydroxide, Sodium-P- nitrophenol, p-nitrophenol, Citric acid, sodium citrate were obtained from Sisco Research Laboratories Pvt. Ltd.

Animals

Female albino rats of Wister strain (150-160g) body weight were used for this study. The animals were obtained from the animal house of Tripura University. The rats were housed in clean and disinfected plastic cages and were fed with a standard rat food and allowed to drink water *ad libitum*. They were maintained in a controlled condition of temperature (25± 2°C) and normal day/night schedule (12L: 12D). The entire study protocol was approved by the Institutional Animal Ethical Committee (IAEC).

Study design

The rats were divided into five groups of six (6) rats in each group. All the animals got their respective treatment orally through gavage.

Group I

(Control Group): Received distilled water 2 ml / day for 90 days.

Group II
(Arsenic treated group): Received arsenic at 38.0 ppm / day / kg bw for 90 days.
Group III
(Lead treated group): Received lead at 22.0 ppm / day / kg bw for 90 days.
Group IV
(Cadmium treated group): Received cadmium at 9.8 ppm / day / kg bw for 90 days.
Group V
(Mixture treated group): Received mixture of heavy metals arsenic, cadmium and lead {38.0ppm (As) + 9.8ppm (Cd) + 22.0ppm (Pb)}/Kg b.w./day for 90 days.
The doses of drugs were determined on the basis of our previous findings on the combined effect of mixture of metals Pb, Cd and As and on reported base line dose of metals in water[18, 19].

Animal Sacrifice
At the end of treatment period, animals were sacrificed by cervical dislocation following ether anaesthesia.Utmost care was taken during the time of sacrifice according to Indian Council of Medical Research (ICMR) guide lines[20].

Body weight and organ weight
The weight of each rat was taken on the first day of the experiment. During the entire period of experiment the weight of the rats were monitored at the interval of fifteen days. The body weight taken on the 1st day of experiment was considered as the initial body weight. The body weight taken on the 91st day before autopsy was considered as final body weight. After autopsy the liver and kidney were dissected out and freed from adherent tissues and blood vessels, blotted free of mucous and weighed to the nearest milligram. The organ weights were expressed per 100g body weight to ensure normalization of data for statistical analysis.

Collection of serum
Blood was collected by puncturing the heart and kept overnight at room temperature for clotting. Serum was collected in serum collecting vials. The serum was stored at - 20^0c until the biochemical analysis.

Collection of organs (Liver& Kidney)
Liver and kidney from the control and experimental animals were quickly excised and washed in ice-cold saline. They were blotted by whatman No.1 filter papers for drying. They were kept at -80^0c until the preparation of permanent slides for the histopathological study.

Preparation of tissue homogenate
Liver and kidney tissue homogenate were prepared according to the procedure of Adekunle et.al[21]. The sample was homogenized by using a Teflon homogenizer in aqueous phosphate, K_2PO_4/$KHPO_4$ buffer (0.1 M ; P^H= 7.4) ; in 4:1 volume buffer to organ weight at 4^0C.

Histopathological study
The liver and kidney were fixed in 4% formalin solution for 48 hrs. The fixed organs were dehydrated in ascending series of alcohol, cleared in xylene and embedded in paraffin wax, and then 4-5 µm thick sections were obtained by rotary microtome and stained by Harris haematoxylin and Eosin. The stained section of the organs was examined under low power (20X) and high power (40X) and photomicrographs were taken.

Biochemical studies from serum and tissue homogenate
To evaluate hepatotoxicity and nephrotoxicity various biochemical parameters were evaluated from serum and tissue homogenates. The serum glucose was measured by the GOD/POD method[22].The serum protein was measured by the Biuret method[23]. The serum albumin was measured by the BCG method [24]. The serum cholesterol was measured by the CHOD/PAP method[22]. The serum urea was measured by the modified Berthelot method[25]. The serum creatinine was measured by the modified Jaffe's Kinetic method[26]. The serum glutamic pyruvic transaminase (SGPT) and the glutamic oxaloacetic transaminase (SGOT) were measured by the Reitman & Frankel's method[27]. Alkaline phosphatase (ALP) and acid phosphatase (ACP) from liver and kidney homogenates were measured by the method of Bessey et.al[28].

Data analysis
All the results were expressed as mean ± S.E. The data were statistically analysed by one – way analysis of variance (ANOVA) followed by Turkey HSD test. Statistical presentations were done by using Vassar stats- one way analysis variance for independent or correlated samples. Inter group comparison were carried out and p values, <0.05 and <0.01 were considered as significant.

RESULT AND DISCUSSION
Table I shows that there was a significant decrease in percentage gain of body weight and a significant increase in weight of liver and kidney in rats treated with a mixture of cadmium, lead and arsenic at a dose of

{38.0ppm (As) + 9.8ppm (Cd) + 22.0ppm)}/Kg bw/day in comparison to all other groups. Chronic exposure to single metal at this low dose did not show any significant variation in body weight gain and weight of liver and kidney in comparison to control animal.

There was a significant increase in the serum levels of glucose, cholesterol, urea and creatinine and a significant decrease in serum levels of protein and albumin in the animals treated with the mixture of cadmium, lead and arsenic **(Table 2).** The serum levels of SGOT and SGPT enzymes in the animals treated with the mixture of metals increased significantly in comparison to all other groups **(Table 2).**

The study showed that there was a significant increase in the activity of enzymes, alkaline phosphatase and acid phosphatase in both liver and kidney in the animals treated with the mixture of cadmium, lead and arsenic in comparison to all other groups **(Fig. 1A , Fig. 1B, Fig. 2A & Fig. 2B)** .

The histological architecture of the liver treated with the mixture of metals showed vacuolation in some hepatocytes, fatty degeneration in other hepatocytes and congestion within central veins and some sinusoids (**Fig. 3C & Fig. 3D)**.The histological architecture of the kidney of rats treated with the mixture included congested glomerulus, degenerated tubule, focal area of coagulative necrosis and exfoliated epithelium (**Fig. 4C & Fig. 4D)**.

Our study revealed that chronic low dose exposure to mixture of heavy metal As, Cd and Pb resulted in general toxic affect in female albino rat, which was evident from decrease in percentage gain in body weight with decrease in food efficacy and water consumption in rats exposed to mixture of metals for 90 consecutive days. Decreased gain in body weight in animals treated with mixture of heavy metal (As, Cd, and Pb) relative to control animal and animal treated with individual metal As, Cd or Pb indicated growth retarding effect of the metals while applied in mixture. Reduction in foodand water intake might contribute to decrease in body weight in exposed animals[29]. Previously studies on concurrent administration of Pb, Cd and As in male rats showed similar decrease in body weight gain and reduced food utilization[30,31].

In agreement with findings of several toxicological studies of heavy metals**,** we observed an increase in weight of both liver and kidney in mixture treated animals. The findings support the view that both liver and kidney are the primary target organs produced toxic effects[32] by chronic low dose exposure to the mixture of these three heavy metals.Treatment with the mixture of metals arsenic (As), cadmium (Cd) or lead (Pb) in rats resulted in a significant increase in serum glucose, cholesterol, urea and creatinine levels and a significant decrease in protein and albumin levels, indicating a systemic toxic effect of the treatment. The increase in serum glucose is a common in heavy metal toxicitywhich is usually connected with inhibition of insulin release from β-cellsand/or a block of glucose utilization by cells even in the presence of increased concentrations of insulin or this might be due to alteration in glucagon secretion resulting into high glycogen breakdown and new supply of glucose produce from other non- carbohydrate substances such as proteins.The increase in serum cholesterol level might be due to the activation of cholesterol synthetic enzymes and simultaneous suppression of cholesterol catabolic enzymes[33]. A significant decrease in serum total protein and albumin levels in animals treated with the mixture of heavy metals (As, Cd and Pb) compared to control animals and animal treated with individual metalsmight be due to changes in protein synthesis and/or metabolism[34].

An increase in serum urea and creatinine level in the animals, treated with mixture of heavy metals (As, Cd and Pb), relative to control animal and animal treated with individual metal As, Cd or Pb indicated nephrotoxic effect of metals treatment. Both urea and creatinine are excreted through kidney. In fact, urea is the first acute renal marker which increases during any kind of kidney injury. But, creatinine is the most trustable renal marker which increases only due to loss of major renal function[35].

Increased activity of serum enzymes such as SGPT and SGOT in animals, treated with the mixture of heavy metals signified the hepatotoxic effect of the metals while applied in mixture, as both SGPT and SGOT enzymes are the hepatic biomarker[36]**.** The result revealed that the degenerative effect of these three heavy metals on the liver cells resulted in the leakage of cytosolic enzymes into circulation. Alkaline phosphatase and acid phosphatase, the important biomarkers of hepatotoxicity and nephrotoxicity showed increased activity in the animals treated with the mixture of heavy metals whichfurther confirmed the hepatotoxic and nephrotoxic effect of the chronic low dose exposure to the metal mixture.

Our histopathological findings of damages to hepatic tissue like vacuolation of hepatocytes with congestion of central vein and sinusoid complements the biochemical findings of hepatotoxic parameters. The histopathology of kidney showed congestion of glomerulus,

degeneration of tubules, focal area of coagulative necrosis and exfoliated epithelium congestion of renal blood vessels. The toxic histopathological changes in liver and kidney in our study after chronic low dose exposure of heavy metals are similar to those reported by various studies on chronic metal toxicity[37,38].

In conclusion, chronic low dose exposure to a mixture of Pb, Cd and As resulted in hepatotoxicity and nephrotoxicity in female rats.

Table I: Effect of treatment with Arsenic (As), Cadmium (Cd), Lead (Pb) & mixture of (As, Cd &Pb) on body weight gain and weight of vital organs (Liver and Kidney)

Parameters	Group-I Or Control Group (06)	Group-II or Arsenic (As) Treated Group (06)	Group-III or Lead (Pb) Treated Group (06)	Group-IV or Cadmium(Cd)Treated Group (06)	Group-V or Mixture of Arsenic, Cadmium & Lead (As, Cd, Pb) Treated Group (06)
Body weight gain (%)	95.25±1.35	86.30±3.58 a*e**	84.37±3.53 b#f**	85.16±3.25 c#g**	63.88±2.44 d**e**f**g**
Weight of the liver (g/100gbw)	3.63±0.01	3.33±0.05 a#e**	4.22±0.43 b#f**	4.52±0.34 c#g**	5.83±0.40 d**e**f**g**
Weight of the kidney (g/100gbw)	0.48±0.03	0.31±0.02 a**e**	0.50±0.008 b#f**	0.50±0.02 c#g**	0.82±0.01 d**e**f**g**

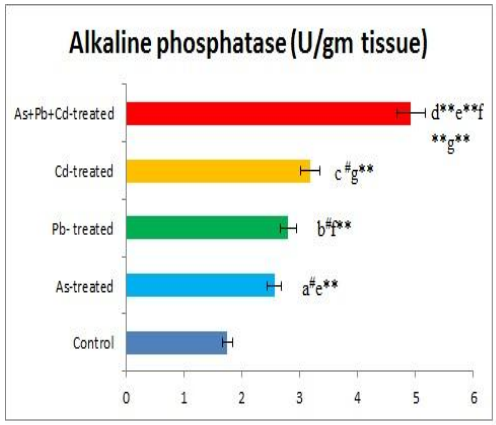
Number in parenthesis indicates no. of animal in each group.
Values represent mean ± SE of six (06) rats; Statistical evaluation by one–way ANOVA followed by HSD comparison; a = Group I vs Group II; b = Group I vs Group III; C = Group I vs Group IV; d = Group I vs Group V; e = Group II vs Group V; f =Group III vs Group V; g = Group IV vs Group V; * = p <0.05; ** = P< 0.01; *** = p<0.001.

Table 2: Effect of treatment with Arsenic (As), Cadmium (Cd), Lead (Pb) & mixture of (As, Cd &Pb) on Serum Glucose, Protein, Albumin, Cholesterol, Urea, Creatinine, SGOT and SGPT of the animals.

Parameters	Group-I Control Group (06)	Group-II Arsenic (As) Treated Group (06)	Group-III Lead (Pb) Treated Group(06)	Group-IV Cadmium(Cd)Treated Group (06)	Group-V Mixture of (As, Cd, Pb) Treated Group (06)
Serum glucose (mg/dl)	108.72±2.47	127.72±10.50 a#e**	109.43±3.31 b#f**	131.30±5.65 c #g**	155.56±3.86 d**e**f**g**
Serum protein (g/dl)	7.49±0.28	6.51±0.29 a#e**	5.32±0.36 b#f**	6.27±0.36 c #g**	3.89±0.16 d**e**f**g**
Serum albumin(g/dl)	4.11±0.03	3.55±0.22 a#e**	3.23±17 b#f**	3.41±0.44 c #g**	1.91±0.11 d**e**f**g**
Serum cholesterol (mg/dl)	65.81±0.94	74.19±1.56 a#e**	69.78±4.91 b#f**	64.37±3.65 c #g**	86.84±0.77 d**e**f**g**
Serum Urea (mg/dl)	45.63±0.01	47.33±0.05 a#e**	55.22±3.65 b#f**	56.52±6.70 c #g**	74.37±3.59 d**e**f**g**
Serum Creatinine (mg/dl)	0.60±0.02	0.69±0.007 a#e**	1.40±0.41 b#f**	1.38±0.51 c #g**	2.88±0.26 d**e**f**g**
SGOT (U/ml)	65.82±2.86	70.03±3.72 a#e**	71.49±3.42 b#f**	72.92±2.41 c #g**	88.72±2.86 d**e**f**g**
SGPT (U/ml)	50.82±2.77	52.46±4.73 a#e**	54.09±4.35 b#f**	59.93±5.69 c #g**	68.22±3.74 d**e**f**g**

Number in parenthesis indicates no. of animal in each group.
Values represent mean ± SE of six (06) rats; Statistical evaluation by one–way ANOVA followed by HSD comparison; a = Group I vs Group II; b = Group I vs Group III; C = Group I vs Group IV; d = Group I vs Group V; e = Group II vs Group V; f =Group III vs Group V; g = Group IV vs Group V; * = p <0.05; ** = P< 0.01; *** = p<0.001.

Fig. 1:



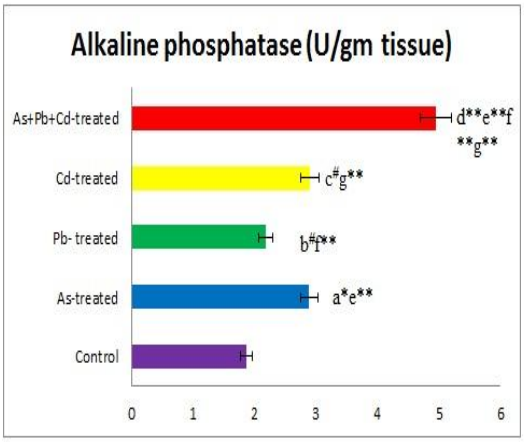
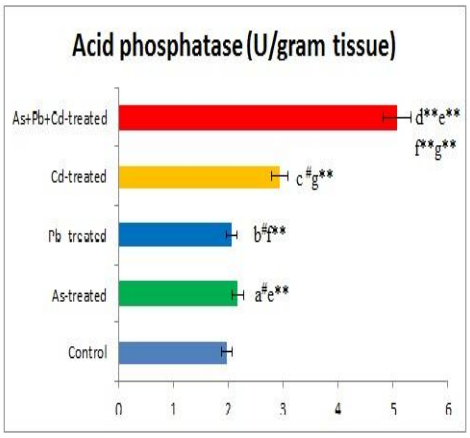


Fig. 1A: **Fig. 1B:**

Fig. 1A & 1B: Effect of treatment with Arsenic (As), Cadmium (Cd), Lead (Pb) & Mixture of (As, Cd &Pb) on Alkaline phosphatase (Unit/gm Tissue) of liver and kidney of the animals. Values represent mean ± SE of six rats; Statistical evaluation by one–way ANOVA followed by HSD comparison; a = Group I vs Group II; b = Group I vs Group III; C = Group I vs Group IV; d = Group I vs Group V; e = Group II vs Group V; f =Group III vs Group V; g = Group IV vs Group V; * = p <0.05; ** = P< 0.01; *** = p<0.001.

Fig. 2:



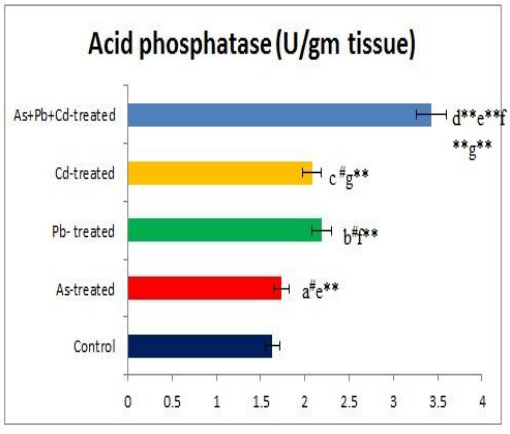


Fig. 2A: **Fig. 2B:**

Fig . 2A &2B: Effect of treatment with Arsenic (As), Cadmium (Cd), Lead (Pb) & Mixture of (As, Cd &Pb) on Acid phosphatase (Unit/gm Tissue) of liver and kidney of the animals. Values represent mean ± SE of six rats; Statistical evaluation by one–way ANOVA followed by HSD comparison; a = Group I vs Group II; b = Group I vs Group III; C = Group I vs Group IV; d = Group I vs Group V; e = Group II vs Group V; f =Group III vs Group V; g = Group IV vs Group V; * = p <0.05; ** = P< 0.01; *** = p<0.001

Fig. 3:

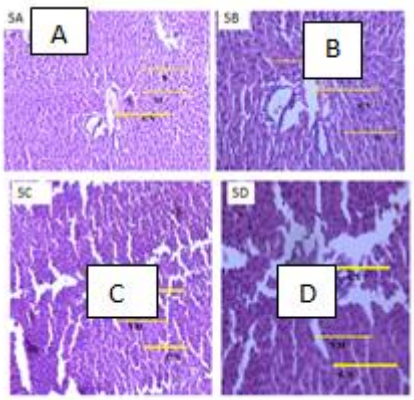


Fig. 3A&3B: Section from control rat liver showing, healthy hepatocytes (H), sinusoids (S) and central vein (CV) (H&E, 20X).
Fig. 3C &3D: Section from treated rat liver showing, vacuolation of epatocytes (VH) with congestion of central vein (CV) and sinusoids (CS) (H&E,40X).

Fig. 4:

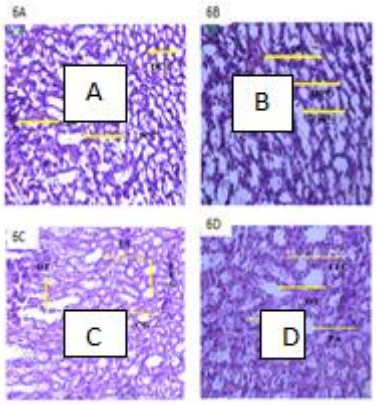


Fig. 4A&4B: Section from control rat kidney showing, normal glomerulus (GI), proximal (PCT) and distal convoluted tubular (DCT) epithelium (H&E, 20X).
Fig.4C &4D: Section from treated rat kidney, showing congested glomerulus (CG), degenerated tubule (DT), focal area (FA) of coagulative necrosis & exfoliated epithelium (EEC)(H&E,40X).

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