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Protective role of s-Adenosyl methionine (SAMe) in cadmium induced toxicopathological changes in Wistar rats

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Abstract

Cadmium is considered to be one of the most toxic substances in the environment due to its wide range of organ toxicity and long elimination half-life. SAMe is recognized as the major methyl-donor for essential methylation reactions that occur in all living organisms. SAMe has been increasingly utilized as a therapeutic agent in liver and neurological disorders recently. The present study has been devised to explore the alleviating effects of SAMe in cadmium induced toxicopathological changes in rats. The rats were divided into four experimental groups: group I formed the control, group II animals were given cadmium chloride @ 200ppm in distilled water, orally, group III animals were given SAMe @ 1 mg/kg body weight, orally and group IV were given both CdCl2 and SAMe. Six rats from each group were humanely sacrificed at 30th, 60th and 90th day. The quantitative data of body weight, absolute organ weight, cadmium content and oxidative stress parameters was analyzed by Duncan's multiple range tests The group II animals showed most of the pathological changes compared to the rest of the group. The magnitude of lesions in various organs was lesser in group IV where SAMe was given. The severity of lesions increased with the advancement of experiment; most severe pathological changes occurring on the 90th day. The level of cadmium residue in liver and kidneys of rats was also found to be increasing with the time in group II and IV; with group IV showing a relatively lesser increase. Similarly the evaluation of oxidative stress parameters reflected the detrimental effect of cadmium in group II and an alleviating effect of SAMe in group IV. Based on the results obtained, SAMe is found to alleviate the toxicopathological effects of cadmium in rats which needs to be further explored.

Keywords: cadmium, S-adenosyl methionine, rats, toxicopathology

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Introduction

According to the Agency for Toxic Substances and Disease Registry (ATSDR), Cadmium is ranked 7th in the 2015 priority list of hazardous substances. This listing is a prioritization of substances based on a combination of their frequency, toxicity, and potential for human exposure. Cadmium is considered to be one of the most toxic substances in the environment due to its wide range of organ toxicity and long elimination half-life as reviewed by Patrick[1] in 2003. Exposure to cadmium occurs mainly by ingestion and inhalation[2] . Cadmium toxicity results in lung, liver, kidney and bone effects as reported by several workers. As in humans, cadmium toxicity has also been reported in animals[3].

S-adenosylmethionine, also known as SAMe or AdoMet, is the second most widely used enzyme substrate after ATP[4]. SAMe is recognized as the major methyl-donor reagent for essential methylation reactions that is essential in diverse processes from fetal development to brain function[5]. S-adenosylmethionine is thought to have a specific role in the alleviation of heavy metal toxicity like cadmium[1]. Not much review is available regarding the alleviating effect of SAMe in cadmium toxicity in

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animals. The following study has been conducted with an aim to explore the therapeutic effects of SAMe on cadmium induced toxicity.

Materials and Methods

Seventy two, 4 weeks old, male Wistar rats weighing between 80-100 grams were obtained from Laboratory Animal Resources Section of the Indian Veterinary Research Institute (IVRI), Izatnagar (U.P). The rats were kept in poly-propylene cages, provided with nutritionally adequate standard diet (Ashirwad Industries Limited, Punjab) and water *ad libitum*. Adequate lighting, ventilation, temperature (21±2^{O}C), relative humidity (50±10 %) and hygienic conditions were maintained throughout the experiment. Toxicity of Cadmium chloride ($CdCl_2$) in Wistar rats was evaluated following approval from Institutional Animal Ethics Committee (IAEC). Prior to study, the rats were dewormed, acclimatized for 15 days, randomly divided into 4 groups each of 18 animals; each group subdivided into 3 subgroups of 6 rats each. Picric acid solution was used to mark the treated animals. The rats of group I were given distilled water and served as control. The rats of group II were intoxicated with cadmium chloride @ 200ppm in distilled water, orally[6,7]. The rats of group III were administered S-adenosyl methionine @ 1 mg/kg body weight, orally[8] and group IV rats were given cadmium chloride@ 200mg/L in distilled water and S-adenosyl methionine @ 1 mg/kg body weight for 30, 60 and 90 days by oral route daily. All the rats were kept under observation to record the clinical manifestations of toxicity. Technical grade cadmium chloride, anhydrous (CAS no. 10108-64-2, Minimum Assay-95%) and S-adenosyl-L- methionine disulfate (C_{22}H34N6O16S4, CAS no. 29908-08-0) used in the study were procured from HiMedia, India.

The body weight of all the experimental rats of group I, II, III and IV was recorded at ten days of interval of experimentation. Absolute weight of liver, lungs, kidneys, heart, brain and spleen, of different groups of rat were determined and expressed in terms of gm. Six rats from each group were humanely sacrificed at the end of study period (on day 30, 60 and 90) and were subjected to detailed post-mortem examination. The representative tissue samples were collected in 10% neutral buffered formalin for histopathological studies. Paraffin embedded tissue sections of 5 µm were obtained and stained with haematoxylin and eosin (H and E) as per the method described by Luna[9] with slight modifications. The stained sections were examined under light microscope and the lesions were recorded, if any.

Estimation of different oxidative stress parameters in liver and kidneys were carried out as described by earlier workers. Feed was withdrawn 24 hr before sacrifice. Liver and kidneys were collected, washed with ice cold normal saline and weighed and stored at -80^{o}C until assayed. Frozen liver and kidney sample were thawed at room temperature and 200 mg of sample was weighed and taken in 2 ml of ice-cold saline for estimation of lipid peroxidation (LPO), superoxide dismutase (SOD), catalase, and total protein. An amount of 200 mg of sample was weighed separately and taken in 2 ml of 0.02 M EDTA for glutathione (GSH) estimation. The homogenates prepared by using homogenizer, under cold conditions were centrifuged for 10 min at 3000 rpm. The supernatant was used for assay of reduced glutathione[10], Catalase[11], lipid Peroxidation[12] and superoxide dismutase[13]. Total protein in liver and kidney homogenate was estimated by the method of Lowry and coworkers[14]. The estimation of various parameters was carried out by double beam UV-VIS spectrophotometer.

For quantitative estimation of cadmium in soft tissues, the biological samples *viz.* liver and kidneys were collected at all the intervals namely at 30, 60 and 90 days and digested as per the procedure described by Kolmer and coworkers[15]. Cadmium content in the digested liver and kidneys samples was measured by using Atomic Absorption Spectrophotometer (AAS 400, Perkin Elmer, USA).

Statistical analysis

The data collected was statistically analyzed for arriving at meaningful conclusion. The quantitative data of body weight, absolute organ weight, cadmium content and oxidative stress parameters was analyzed by Duncan's multiple range tests[16] .

Results

Clinical signs, body and organ weight

The rats of group-II showed overt clinical signs like inappetance, reduced water intake, dry skin, rough hair coat, dullness, depression, emaciation and decrease in growth rate. The clinical signs showed by the rats got more pronounced with the advancement of experiment. The

rats of group-IV exhibited similar signs as that of group-II but of reduced intensity. And those belonging to group-I (control) and III appeared active and healthy throughout the experimental period. The body weight of rats in group II was reduced ($p<0.001$) as compared to group I and III rats. Moreover, the body weight of rats of group IV did not differ significantly but showed improvement with respect to the rats of group II. In general, there was reduction in the mean values of absolute weight of various organs viz. liver, lungs, kidneys, heart, brain, spleen and testes in the rats of group-II that were given $CdCl_2$.

Gross findings

The morbid lesions in between the toxicity groups were found to be severe in group-II as compared to group-IV. The rats of group-I and group-III showed no significant pathomorphological lesions in different organs. Grossly, the liver of rats of all the groups did not manifest any gross lesion at 30 days of interval. Pallor with occasional pinpoint haemorrhages and mottling on the dorsal surface was observed on 60th day onwards. The lungs of rats of group-II and group-IV were congested and edematous at various time intervals. The lungs of the rats from group II showed small to large grayish white nodules protruding on the surface on day 90 (fig 1). The kidneys of rats of group-II and group-IV revealed no significant changes except mild congestion at various intervals. The epicardial surface of the heart showed mild congestion with occasional petechiae in the rats of group-II on day 60 and onwards. No significant lesions were recorded in the rats of group-IV. The small intestine of rats of group-II showed mild thickening in the intestinal wall. The brain of the toxicity groups showed mild meningeal congestion. The testes of the rats of group-II showed mild congestion.

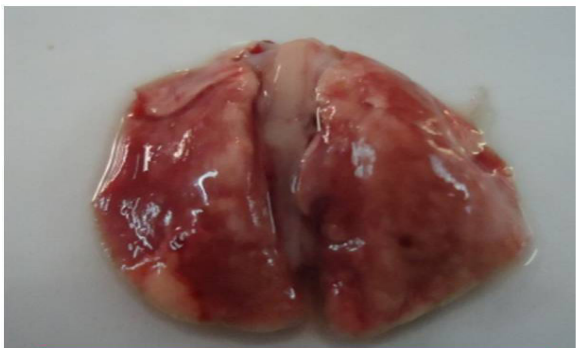


Figure 1. Lungs from the rats of group II on day 90 showing small to large grayish white nodules protruding from the surface.

Histopathological findings

The rats of group I and III showed no significant histopathological lesions. The grades of microscopic lesions in group II was more than those in group IV. Also the severity of microscopic changes increased with time with more lesions on 90th day in group II. The magnitude of increase in lesions in group IV was lesser than that in group II showing a reparative effect of SAMe. The lungs of three rats of group-II on day 90 also revealed the lesions of pneumocyto-carcinoma featured by presence of large, flat, pleomorphic squamous type of cells filling the alveolar lumen having hyperchromatic nuclei (Fig.2). Some of the proliferated pneumocytes showed degeneration and desquamation. There was a focal lesion of encephalomalacia with aggregation of microglia in a few rats of group-II on day 90 (fig 3). Mild depletion spermatogonial cells of semniferous tubules of testes was also noted in rats of group II on day 60 (fig 4).

In group IV, heart, kidneys, intestines, brain and testes showed the same grade of lesions throughout the study with no increase.

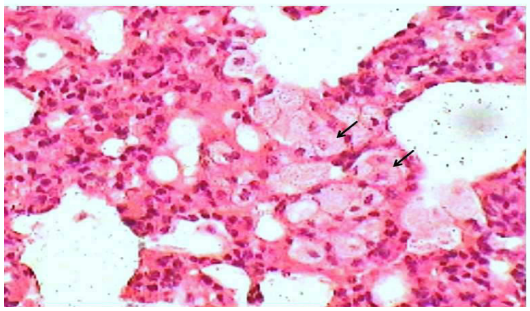


Figure 2. Section of lungs from the rats of group-II showing presence of large flat pleomorphic cells with hyperchromatic nuclei (arrows) on day 90. (H&R 400X).

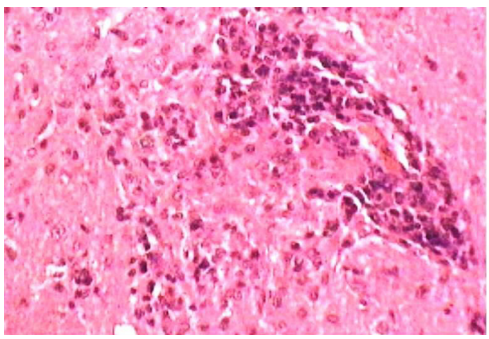


Figure 3. Section of brain showing focal area of encephalomalacia with infiltration of microglia cells in the rats of group-II on day 90. (H&E 400X).

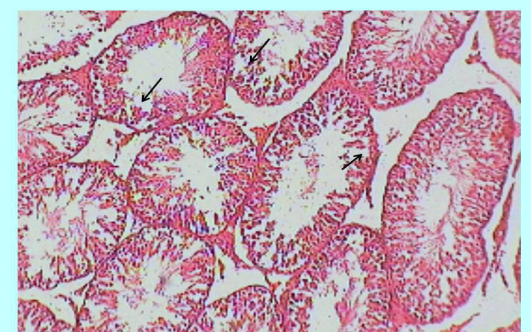


Figure 4. Section of testes showing depletion of spermatogonial cells (arrows) in the rats of group-II on day 60. (H&R 100X).

Oxidative stress parameters

The mean values of SOD, catalase, LPO and GSH in liver and kidney homogenate on day 30, 60 and 90 are presented in **table 1** and **2** respectively. There was highly significant (P<0.01) decrease in the mean values of SOD in liver homogenate in the rats of group-II in all the intervals of experimentation. Similar decrease was noted in group-IV as compared to group-I and III at 60 and 90 days of intervals (table 1). There was significant (P<0.05) decrease in the mean values of SOD in kidney homogenate in group-IV at 30 days of interval and highly significant (P<0.01) decrease at 60 and 90 days as compared to group-I and group-III (table 2).

The statistical analysis indicated highly significant (P<0.01) increase in the mean values of catalase in liver homogenate in rats of group-II and IV as compared to group-I and III at 30 and 60 days of intervals and highly significant (P<0.01) increase in the mean values of catalase in liver homogenate in rats of group-II as compared to group-I, III and IV at 90 days of interval. The statistical analysis revealed highly significant (P<0.01) increase in the mean values of catalase in kidney homogenate in rats of group-II as compared to group-I, III and IV at all the intervals of the experiment.

The statistical analysis revealed highly significant (P<0.01) increase in the mean values of LPO in liver and kidney homogenate in rats of group-II as compared to group-I, III and IV at all the intervals of the experiment.

There was highly significant (P<0.01) decrease in the mean values of GSH in liver and kidney homogenate in the rats of group-II in between all the intervals of experimentation. A decrease in the mean values of GSH in liver and kidney homogenate in the rats of group IV was also observed but not as much as in group II.

Estimation of cadmium in soft tissues

The mean tissue cadmium residue in the liver and kidneys of rats from different groups at 30, 60 and 90 days interval is depicted in **table 3**. The statistical analysis revealed significant (P<0.05) increase in the mean cadmium concentration in the liver in rats of group-II as compared to group-I, III and IV on day 30 and 60. There was significant (P<0.05) increase in the mean cadmium concentration in the liver in the rats of group-II in between all the intervals of experimentation.

Table 1. Mean values of SOD, catalase, LPO and GSH in liver of rats of different groups at various intervals.

Period (days)	SOD (U/mg of protein)				Catalase (mM H_2O_2 utilized/min/mg of protein)				LPO (nM MDA formed/ gm of wet tissue)				GSH (mM GSH/ gm of wet tissue)			
	G-I	G-II	G-III	G-IV	G-I	G-II	G-III	G-IV	G-I	G-II	G-III	G-IV	G-I	G-II	G-III	G-IV
30	29.	19.	30.	26.	59.	68.	55.	65.	64.	81.	60.	73.	0.	0.	0.	0.
	79^{Aa}	87^{Ba}	07^{Aa}	91^{Aa}	28^{Ba}	80^{Ac}	21^{Bb}	65^{Ac}	03^{Ca}	54^{Ac}	00^{Db}	01^{Bc}	082^{Aa}	072^{Ba}	083^{Aa}	076^{Ba}
	±1.	±0.	±0.	±0.	±1.	±1.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.
	13	54	95	35	47	25	97	89	45	49	72	89	001	001	001	001
60	28.	16.	30.	19.	60.	73.	57.	69.	65.	90.	63.	81.	0.	0.	0.	0.
	93^{Aa}	05^{Cb}	73^{Aa}	39^{Bb}	90^{Ba}	42^{Ab}	00^{Cab}	94^{Ab}	67^{Ca}	94^{Ab}	01^{Da}	47^{Bb}	082^{Aa}	062^{Cb}	082^{Aa}	070^{Bb}
	±1.	± 0.	±0.	±0.	±1.	±1.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.
	24	36	59	00	04	20	68	65	52	82	74	45	002	002	002	002
90	29.	13.	28.	18.	61.	84.	59.	74.	65.	107.	63.	92.	0.	0.	0.	0.
	76^{Aa}	35^{Cc}	73^{Aa}	40^{Bb}	66^{Ca}	62^{Aa}	68^{Ca}	40^{Ba}	28^{Ca}	88^{Aa}	03^{Ca}	76^{Ba}	081^{Aa}	054^{Cc}	081^{Aa}	066^{Bb}
	±1.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±1.	±0.	±0.	±0.	±0.	±0.	±0.
	04	28	85	34	86	77	70	59	59	44	72	74	002	002	001	002

Mean with different superscript (A, B, C) in rows are differing significantly in between the groups, otherwise non-significant.

Mean with different superscript (a, b, c) in a columns are differing significantly in between the intervals, otherwise non-significant.

Table 2. Mean values of SOD, catalase, LPO and GSH in kidneys of rats of different groups at various intervals.

Period (days)	SOD (U/mg of protein)				Catalase (mM H_2O_2 utilized/min/mg of protein)				LPO (nM MDA formed/gm of wet tissue)				GSH (mM GSH/ gm of wet tissue)			
	G-I	G-II	G-III	G-IV	G-I	G-II	G-III	G-IV	G-I	G-II	G-III	G-IV	G-I	G-II	G-III	G-IV
30	26.	19.	27.	22.	41.	51.	40.	45.	57.	73.	52.	64.	0.	0.	0.	0.
	33^{Aa}	01^{Ca}	51^{Aa}	04^{Ba}	67^{Ca}	71^{Ac}	55^{Ca}	41^{Bc}	89^{Ca}	40^{Ac}	04^{Da}	82^{Bc}	066^{Aa}	056^{Ba}	066^{Aa}	062^{ABa}
	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.
	68	50	74	50	65	77	61	52	70	69	79	36	001	002	002	001
60	26.	14.	26.	19.	42.	61.	41.	54.	58.	85.	53.	74.	0.	0.	0.	0.
	62^{Aa}	89^{Cb}	85^{Aa}	47^{Bb}	81^{Ca}	25^{Ab}	66^{Ca}	05^{Bb}	65^{Ca}	90^{Ab}	19^{Da}	33^{Bb}	065^{Aa}	049^{Bb}	067^{Aa}	054^{Bb}
	±0.	±0.	±0.	±0.	±0.	±1.	±0.	±0.	±0.	±0.	±0.	±0.	± 0.	±0.	±0.	± 0.
	54	49	47	51	42	01	37	44	30	43	46	63	001	002	001	002
90	26.	13.	26.	17.	43.	70.	42.	60.	59.	100.	52.	88.	0.	0.	0.	0.
	04^{Aa}	50^{Cb}	54^{Aa}	58^{Bc}	00^{Ca}	66^{Aa}	08^{Ca}	61^{Ba}	47^{Ca}	32^{Aa}	56^{Da}	14^{B}	066^{Aa}	039^{Cc}	067^{Aa}	050^{Bb}
	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	±0.	± 0.
	45	54	55	38	77	71	73	83	29	93	75	48	001	001	001	001

Mean with different superscript (A, B, C, D) in rows are differing significantly in between the groups, otherwise non-significant.

Mean with different superscript (a, b, c) in a columns are differing significantly in between the intervals, otherwise non-significant.

There was significant ($P<0.05$) increase in the mean cadmium concentration in the kidneys in the rats of group-II as compared to group-I and III on day 60 and 90. In the rats of group-II highly significant ($P<0.01$) increase in the mean cadmium concentration in the kidneys was observed in between all the intervals of experimentation. No significant variation in the mean cadmium concentration was observed in the kidneys and liver in rats of group-I and III in between all the intervals of the experiment.

Discussion

Cadmium has been shown to be toxic to all the biological tissues. It has been reported that Cd causes morphological and functional damage in testicular tissue [17], hepatic[18] and renal tissue[19]. The overt clinical signs and reduction in body weight noticed in group II and IV rats in particular indicate the effect of toxicant and the stress associated with the toxicity. The observed reduction in body weight in toxicity groups could have been due to decrease in feed consumption. An invariable reduction in the mean values of absolute weight of various organs in the rats of group-II is in accordance to the findings of Dzobo and Naik[20]. The absolute organs weight in rats of group-IV administered S-adenosyl methionine showed slight improvement in the weight of organs and were near to that of control group suggesting a protective role of S-adenosyl methionine on paranchymatous organs.

Liver is the main organ concerned with the metabolism of xenobiotics and various toxic insults. Liver is the major target organ of toxicity following acute Cd poisoning, and Cd hepatotoxicity is the major cause of acute Cd lethality[21]. Cadmium is detoxified in the liver through formation of metallothionein-cadmium complex which after release from the liver is readily filtered through the glomerulus and reabsorbed in proximal tubular segments[22]. The congestion, hemorrhage and necrotic changes in liver parenchyma may be attributed to the fact that cadmium has affinity to this organ. Also, cadmium could induce lipid peroxidation in tissues leading to hepatic necrosis which is clearly depicted by the level of oxidative stress parameters in this study. Renal damage is believed to occur due to the intracellular localization of an excessive concentration of cadmium as metallothionein. The degenerative changes in the heart muscle, testis and brain may be due to increase in formation of free radicals and lipid peroxidation. The finding of pneumocyto-carcinoma in three rats in the present study at 90th day of exposure indicates and supports earlier observation for the carcinogenic potential of cadmium in rats[23,24]. S-Adenosyl methionine has been called as "the liver's super-nutrient." SAMe is thought to be important in maintaining cell membrane integrity and act as a key

intermediate in the hepatic transsulfuration pathway. The subtle pathomorphological lesions noticed in this study, in the rats of group-IV as compared to those of group-II rats suggested biological protective role of SAMe.

Studies by earlier workers have shown that cadmium inhibits the activity of majority of enzymes involved in degradation of activated oxygen species inducing an increased production of free radicals, lipid peroxidation, and destruction of cell membranes[25] which clearly explains the severity of lesions in group II where cadmium chloride alone was given. Cadmium also decreases the activities of many enzymes by binding to their sulfhydryl groups or by inhibiting the protein synthesis[26,27]. These findings correlate with our findings here namely a reduction in levels of SOD and GSH in liver and kidneys of cadmium chloride treated rats. At the same time an improvement in the levels of SOD and GSH in group IV was also observed suggesting the ameliorative effect of SAMe in cadmium induced liver injury. SAMe serves as a precursor for glutathione[28,29] (GSH) which serves as a free radical scavenger. In both animal models and patients with liver disease, exogenous administration of SAM raised GSH levels and in animals, ameliorated liver injury significantly[30,31]. But here a significant increase in the mean values of catalase and LPO in liver and kidney homogenates of rats in group II and IV was noted. Sarkar and coworkers[32] have reported a significant increase in catalase activity in heart 9 h after cadmium injection in rats with no significant change in in kidney and liver tissues. Catalase present in the peroxisomes is responsible for the breakdown of hydrogen peroxide formed by the reaction catalysed by SOD. A similar increase in LPO was observed in liver and kidneys of rats treated with cadmium[33]. Previously, Mueller[34] reported that single-dose Cd administration increased lipid peroxidation and decreased GSH in the liver.

Cadmium has special affinity for hepatic and renal parenchyma[35]. Accumulation of cadmium in liver and kidneys in cadmium administered rats has been reported by many workers[33,36,37]. The level of cadmium residue in liver and kidneys of rats was found to be increasing with the time in group II and IV. But the magnitude of increase in tissue calcium residue was much less in group IV where SAMe was also given as compared to the group II where cadmium chloride alone was given. This finding further reinforces the ameliorative potential of SAMe in injured liver.

From the present study it can be concluded that chronic dietary intake of Cd in rats leads to its accumulation in liver and kidney and is linked with distinct alterations in enzymes of oxidative stress. As evident from the results, lipid peroxidation could be associated with cadmium induced toxicopathology in liver and kidney. So also the result shown by the group IV animals clearly backs the fact that SAMe could be used as a therapeutic agent. Although SAMe's beneficial effects appear well-accepted, the exact molecular mechanisms of its protection remain largely ill-defined since it has impact on so many critical cellular processes[29]. Therefore, it is concluded that SAMe could shield the toxic effects of cadmium on biological system which needs to be further explored.

Acknowledgement

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Conflict of Interest

None Declared

Table 3. Mean tissue cadmium residue in liver and kidneys in the rats of different groups at various intervals.

Period (Days)	Liver (µg/gm)				Kidneys (µg/gm)			
	G-I	G-II	G-III	G-IV	G-I	G-II	G-III	G-IV
30	0. 26^{Ca} ±0. 02	0. 46^{Ac} ±0. 02	0. 24^{Ca} ±0. 02	0. 37^{Bc} ±0. 02	0. 30^{Ba} ±0. 02	0. 48^{Ac} ±0. 01	0. 29^{Ba} ±0. 02	0. 44^{Ab} ±0. 01
60	0. 28^{Ca} ±0. 02	0. 53^{Ab} ±0. 02	0. 28^{Ca} ±0. 02	0. 46^{Bb} ±0. 01	0. 31^{Ca} ±0. 02	0. 57^{Ab} ±0. 02	0. 31^{Ca} ±0. 02	0. 50^{Bb} ±0. 01
90	0. 28^{Ba} ±0. 03	0. 64^{Aa} 0. 02	0. 28^{Ba} ±0. 02	0. 56^{Aa} ±0. 02	0. 31^{Ca} ±0. 02	0. 67^{Aa} ±0. 02	0. 31^{Ca} ±0. 02	0. 58^{Ba} ±0. 02

Mean with different superscript (A, B, C) in rows are differing significantly in between the groups, otherwise non-significant.
Mean with different superscript (a, b, c) in columns are differing significantly in between the intervals, otherwise non-significant.

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