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Full Length Research Paper

A comparative study on the biochemical effect of ocular and oral cadmium administration in rabbits

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The objective of the present study was to compare the effect of oral and ocular exposure to cadmium for one and three months on some biochemical parameters using rabbits as animal model. The results obtained show that the kidney, femoral muscle, femur and brain of rabbits ocularly exposed to Cd had a significantly higher concentration of Cd relative to that of those administered the metal orally after both periods, except for the liver which was significantly lower. There was a significantly higher membrane lipid peroxidation (LPO) in the kidney of the one and three months exposed rabbits after both oral and ocular exposure to Cd relative to control with a corresponding decrease in the activities of superoxide dismutase (SOD) and catalase (CAT). Irrespective of the route and time of exposure to the metal, the activity of SOD and CAT were significantly higher in the brain relative to the control, but the level of LPO was not significantly altered. Liver damage induced by oral cadmium after one and three months exposure was clearly shown by the increased activities of plasma hepatic marker enzymes namely, L-aspartate aminotransferase activity (L-AST), L-alanine aminotransferase activity (L-ALT), alkaline phosphatase activity (ALP) along with increased level of LPO indices and a corresponding decrease in the activities of SOD and CAT in the liver. Similarly, liver damage induced by ocular exposure to Cd for one month was manifested as increased plasma L-AST, L-ALT and ALP activities with an increased LPO and a corresponding decrease in the activities of SOD and CAT in the liver. However, the plasma parameters did not significantly change after three months of exposure to ocular Cd, except plasma ALP activity which remained significantly higher. The liver LPO was also not significantly different from the control after three months exposure to ocular Cd, although the SOD and CAT activities significantly decreased. Thus the major finding of the present study was that in relation to ocular exposure, oral exposure to Cd was more toxic to the liver at the end of three months of exposure.

Key words: Cadmium, rabbits, antioxidant enzymes, lipid peroxidation, liver function.

INTRODUCTION

Cadmium (Cd) is among the heavy metals that have main threats to human health. This metal which is widely distributed in the environment due to its use in industry, become toxic when it is not metabolized, which allows it to accumulate in many organs (Jarup et al., 1998; Trinchella et al., 2006). However, the amount of Cd which is distributed to the organs depends on the interval of

exposure, the dose administered, the production and reproduction phase of the animals, as well as their age and breed (Baykov et al., 2003). The liver is one of the critical target organs in chronic Cd exposure (WHO, 1992). Oxidative stress, an important mechanism associated with toxic effects of Cd, has been implicated in liver injury associated with this metal (Asagba et al., 2004; Borges et al., 2008). Besides, Cd can induce and bind to metallothionein (MT), which has been implicated in its detoxification (Waalkes et al., 2004; Klaassen et al., 2009). Thus non-MT bound metal changes the enzyme activity and membrane structure by reacting with the sulfhydryl group of the membrane, resulting in liver injury (Foulkes, 1982). Cd-induced injury in the liver can cause the release of abnormal quantities of L-aspartate amino-

Abbreviations: LPO, Lipid peroxidation; SOD, superoxide dismutase; CAT, catalase; L-AST, L-aspartate aminotransferase activity; L-ALT, L-alanine aminotransferase activity; ALP, alkaline phosphatase activity; Cd, cadmium; MT, metallothionein; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde.

Table 1. Experimental design and the associated parameters.

Experimental design	Parameters
Number of groups	Two
Number of rabbits per group	Twenty four
Designation of group I	Oral Cd exposure
Designation of group II	Ocular Cd exposure
Treatment of oral Cd exposed rats	5 mg Cd/Kg administered orally by gavage 3 times a week
Treatment of ocular Cd exposed rats	5 mg Cd / Kg administered as eye drops 3 times a week.
Number of subgroups per group	Two
Number of rabbits per subgroup	Twelve (six control rabbits and six test rabbits)
Exposure duration (subgroup I)	One month
Exposure duration (subgroup II)	Three months

transferase activity (L-AST) and alanine amino-transferase (L-ALT) into the blood (Asagba et al., 2007; Asagba and Eriyamremu, 2007; Borges et al., 2008). Plasma alkaline phosphatase activity (ALP) activity is another marker for liver damage since the liver isoform is one of the predominant forms of the enzyme which is present in the plasma (Annino and Giese, 1976).

The toxicity of Cd on biological systems of animals depends on the route or mode of administration. Available reports indicate that exposure to Cd can occur through the oral and inhalation routes (WHO, 1992). Exposure to heavy metals (Cd inclusive) via the ocular route may also occur from the general environment (Grubb et al., 1985; Imbrasienne et al., 2007), cigarette smoke (Avunduk et al., 1997) and facial cosmetics (Nnorom et al., 2005). Consequently, the effects of Cd and other chemicals on ocular tissues have been studied by several investigators using animal models. In these studies, ocular exposure to heavy metal salts or other chemicals were either by intravitreal injection (Mayasaka et al., 2001; Bakri et al., 2008) or solutions which were applied as eye drops (Monrad et al., 2005; Eriyamremu et al., 2008a, 2008b). Ocular exposure to Cd may lead to accumulation of these metals in other organs through the systemic circulation. Unfortunately relatively few studies have addressed the effect of ocularly exposed Cd on other organs, and fewer still have compared the biochemical effect of ocular exposure to Cd and that of other routes of exposure. The objective of the present study is to compare the effect of oral and ocular exposure to Cd on some biochemical parameters using rabbits as animal model.

MATERIALS AND METHODS

Animals and experimental design

The animals used for the study were male New Zealand rabbits weighing between 1380 - 1520 g. They were purchased from a farm in Benin City, Nigeria. The animals were maintained on normal chow (Product of Bendel Feed & Flour Mills, Ewu, Edo State, Nigeria) and water *ad libitum* throughout the duration of the study.

After a period of 2 weeks acclimatization to laboratory conditions, they were weighed and distributed into two major groups with 24 animals per group. While one group was exposed to Cd orally, the other was exposed to Cd ocularly. Each of these groups was further divided into two subgroups with twelve animals each (Table 1). Six of the rabbits in each of the subgroups for the oral Cd exposed rabbits received 5.0 mg Cd/kg body weight orally thrice a week, while the other six served as the control and were similarly treated with same volume of saline. While one subgroup was given this treatment for one month, the other received the treatment for three months.

Half of the animals in the subgroups for ocular Cd exposed rabbits received same dose of Cd thrice a week as eye drops while the remaining which served as controls received same volume of saline through the same route thrice a week. Similarly, while one subgroup was given this treatment for one month, the other received the treatment for three months. Animals in all the subgroups were weighed once a week throughout the study period and the feed intake and fecal output were measured daily. At the end of the specified duration of exposure, all the rabbits were weighed and subsequently anesthetized (in chloroform saturated chamber). While under anesthesia, each rabbit was sacrificed by heart puncture by means of hypodermic syringe and needle. In all these treatments, the animals were handled in accordance with the principles of laboratory animal care as contained in National Institutes of Health (NIH) guide for laboratory animal welfare (National research council, 1996). The blood collected from the heart was transferred to heparinized tubes which were carefully swirled. Plasma was later obtained by centrifugation at 3000 g for 10 min and immediately used for biochemical analysis. The liver, kidney, femur, femoral muscle and brain were also quickly excised and portions were used for both metal and biochemical analysis after weighed sections of these organs were homogenized with 0.25 M sucrose.

Metal analysis

The tissue homogenates were digested with nitric acid over night and the concentrations of Cd were analysed using flame atomic absorption spectrophotometry (AAS; Z-5000 polarized Zeeman atomic absorption spectrophotometer, Hitachi, Tokyo, Japan). To verify the Cd content of the tissues, certified standard reference materials, bovine liver 1577b (NIST, USA) and animal bone H-5 (IAEA, Austria) were analysed by the same procedure as the samples from the experiments. The value of Cd in bovine liver was 0.55 µg/g (certified value was 0.50). Cd in animal bone was 0.026 µg/g (reference value was 0.023).

Biochemical analysis

L-ALT and L-AST activities in plasma

The activities of L-ALT (EC.2.6.1.2) and L-AST (EC.2.6.1.1) were determined colorimetrically according to standard procedures using commercially available diagnostic kits (Randox laboratories limited, England). The activities of these enzymes are expressed in units / ml.

ALP activity in plasma

The activity of ALP (EC.3.1.3.1.) was determined colorimetrically using the synthetic substrate p-nitrophenyl phosphate as outlined by Annino and Giese (1976). The activity of the enzyme is expressed in units/ml and one unit is equivalent to one micromole p-nitrophenol produced per min.

Superoxide dismutase (SOD) activity in tissues

The activity of SOD (EC1.15.1.1) in the liver, kidney and brain homogenates was determined by following the autooxidation of epinephrine as described by Misra and Fridovich (1972). The activity of the enzyme is expressed in units/g tissue and one unit of SOD activity is defined as the amount of the enzyme required for 50% inhibition of the oxidation of epinephrine to adrenochrome in one minute.

Catalase (CAT) activity in tissues

The activity of CAT in the liver, kidney and brain homogenates was determined by the method of Kaplan and Groves (1972) and was based on residual H_2O_2 after incubation with the samples; each catalase unit specifies the relative logarithmic disappearance of hydrogen peroxide per min and is expressed as $K \text{ min}^{-1}$, where K is the rate constant for a first order reaction kinetics.

Determination of membrane lipid peroxidation (LPO)

Estimation of membrane LPO in the liver, kidney and brain was done by the method of Gutteridge and Wilkins (1982). The procedure involved the determination of thiobarbituric acid reactive substances (TBARS) which are indicators of membrane LPO. Values for TBARS are reported as malondialdehyde (MDA) and quantitated using a molar extinction coefficient of $1.5 \times 10^5 \text{ M/cm}$ and expressed as units per gramme of tissue weight (one unit is equivalent to one micromole MDA).

Statistical analysis

Statistical analysis of results was performed using the Mann-Whitney non-parametric U-test. The level of significance was $p < 0.05$. All statistical calculations were done with the STATISTICA 5.0 computer programme.

RESULTS

The body weight gain, feed consumption, feed efficiency and fecal output data of Cd exposed rabbits are presented in Figure 1. Rabbits exposed to Cd orally for

one month gained significantly lower body weight than controls, although their feed consumption rate was significantly higher. Rabbits orally treated with Cd also had lower feed efficiency and higher fecal output relative to rabbits treated with Cd free water. Rabbits exposed to Cd ocularly for one month through eye drops also consumed significantly higher feed than controls, although body weight gain, feed efficiency and fecal output were comparable for both groups. Irrespective of the mode of exposure, no significant change was observed in these parameters after three months of exposure. Thus the study shows that alteration in body weight gain, feed consumption and fecal output may be early indicators of Cd toxicity.

Figure 2 presents the Cd contents of the organs of rabbits after oral and ocular exposure to the metal. Irrespective of the mode and duration of exposure, the Cd content of the organs of rabbits was significantly higher than the control. Most Cd was deposited in kidneys and liver, while the least levels were noted in brain and muscle tissues. The kidney, femoral muscle, femur and brain of rabbits ocularly exposed to Cd had a significantly higher concentration of Cd relative to that of rabbits offered the metal orally after both periods of exposures, except for the liver which was significantly lower. Also, irrespective of the mode of exposure, more Cd was accumulated in the tissues of the three months exposed rabbits. Thus the study reveals that the accumulation of Cd in the organs of rabbits depends on the tissue and duration of exposure.

Effects of oral and ocular cadmium exposures on membrane LPO of liver, kidney and brain of rabbits, along with the activities of antioxidant enzymes in these organs are shown in Figure 3. There was a significantly higher level of membrane LPO in the liver and kidney of the one month exposed rabbits after both oral and ocular exposure to cadmium relative to control, with a corresponding decrease in the activities of SOD and CAT. However, the effect of Cd on the activity of liver antioxidant enzymes and level of membrane LPO was more pronounced after oral than after ocular exposure to the metal. Conversely, in the kidney, the data obtained indicated that the effect of Cd on these parameters was more pronounced after ocular than after oral exposure. The activities of SOD and CAT were significantly higher in the brain of one month Cd exposed rabbits, but the level of LPO was not significantly altered. The pattern of results obtained in the one month exposed rabbits was altered in those rabbits similarly exposed to Cd for three months. No significant change was observed in the liver membrane LPO relative to the control after ocular exposure to Cd for three months, but the level of this parameter was significantly higher after oral exposure to Cd in rabbits. The activities of liver SOD and CAT were significantly lower relative to controls after both modes of exposure to Cd; however the effects of Cd on these enzymes were greater after oral exposure. There was a

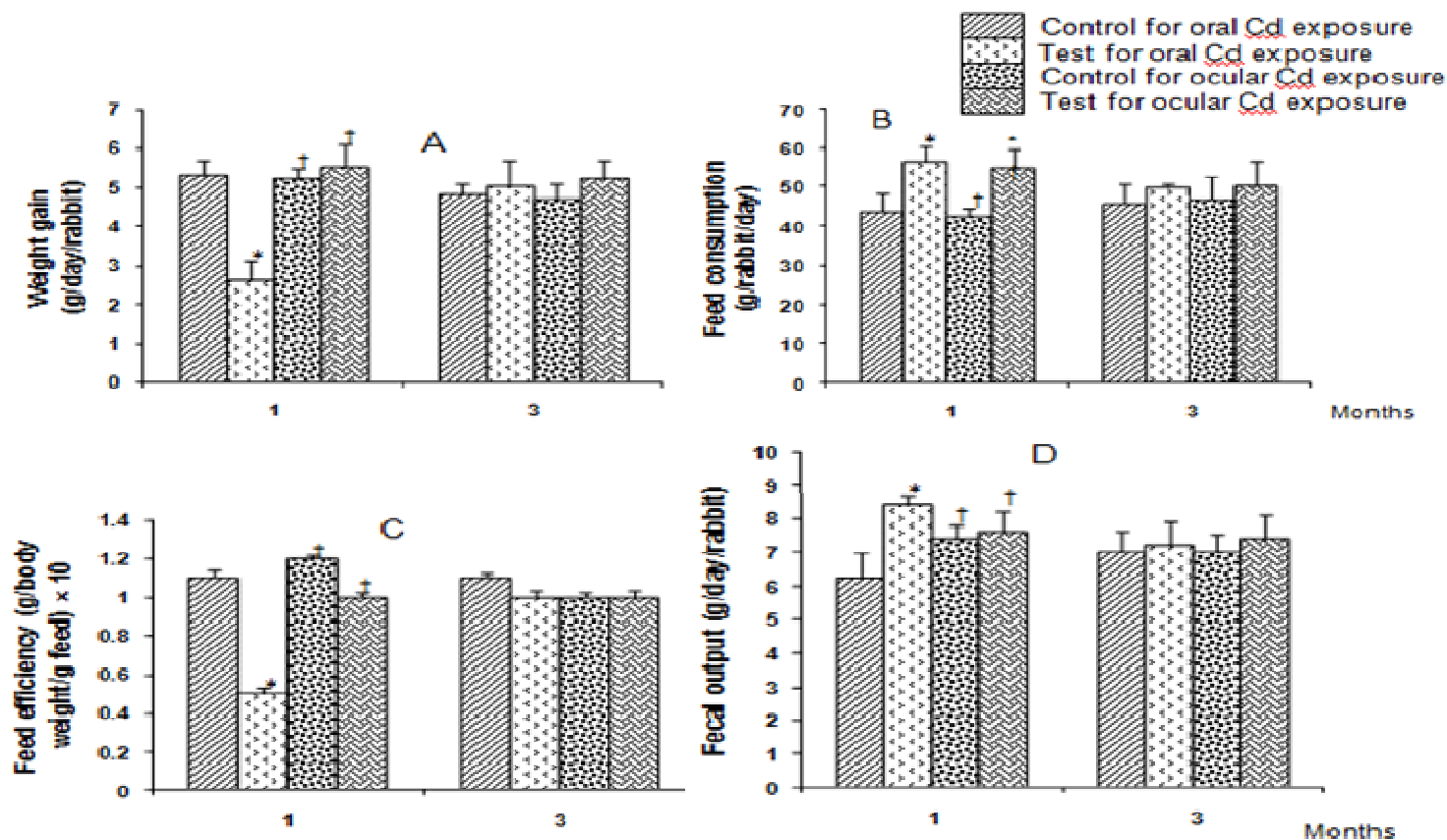


Figure 1. Weight gain, feed consumption, feed efficiency and faecal output of rabbits after oral and ocular exposure to cadmium. Values are means \pm SEM. $P < 0.05$ (Mann – Whitney U – test) compared to control of oral Cd exposure (*), test of oral Cd exposure (†) and control of ocular Cd exposure (‡) groups.

significantly higher membrane LPO and a corresponding lower SOD and CAT activities in the kidney of rabbits after oral and ocular exposure to Cd for three months. Unlike in the liver, the effect of Cd on the activities of the antioxidant enzymes was more pronounced after ocular exposure. Like in the one month exposed rabbits, no significant difference was observed in brain membrane LPO of rabbits exposed to Cd by ocular and oral routes for three months despite the significant inhibition of SOD and CAT activities relative to control. The study shows that the effect of Cd on membrane LPO and activities of SOD and CAT in the tissues of rabbits depends on both mode and duration of exposure.

The liver function parameters of rabbits after both modes of exposures to cadmium are presented in Figure 4. The plasma L-ALT and L-AST activities of rabbits were significantly higher after ocular and oral exposures to cadmium for one month, although the effect of Cd on these enzymes was more pronounced in rabbits exposed to Cd via the oral route. A similar trend was also observed for the plasma ALP activity after both routes of exposure. The plasma ALP activity was significantly elevated in rabbits exposed to Cd for one month via both

routes of exposure with rabbits exposed orally to Cd having a higher activity of the enzyme. The profile of the activities of these enzymes was also altered in rabbits exposed for three months. No significant change in activity of these plasma enzymes was observed in rabbits exposed to the metal via the ocular route relative to control, except for ALP activity which was significantly higher than the control. On the other hand, rabbits treated orally with Cd for three months had significantly elevated ALT, AST and ALP activities relative to controls. The activities of these enzymes were also significantly greater than those of rabbits ocularly treated with the same dose of Cd.

DISCUSSION

The objective of the present study is to compare the effect of oral and ocular exposure to Cd for one and three months on some biochemical parameters using rabbits as animal model. The significantly higher feed intake of rabbits after both route of exposure (Figure 1B) could be attributed to the toxic effect of Cd. Cd intoxication leads

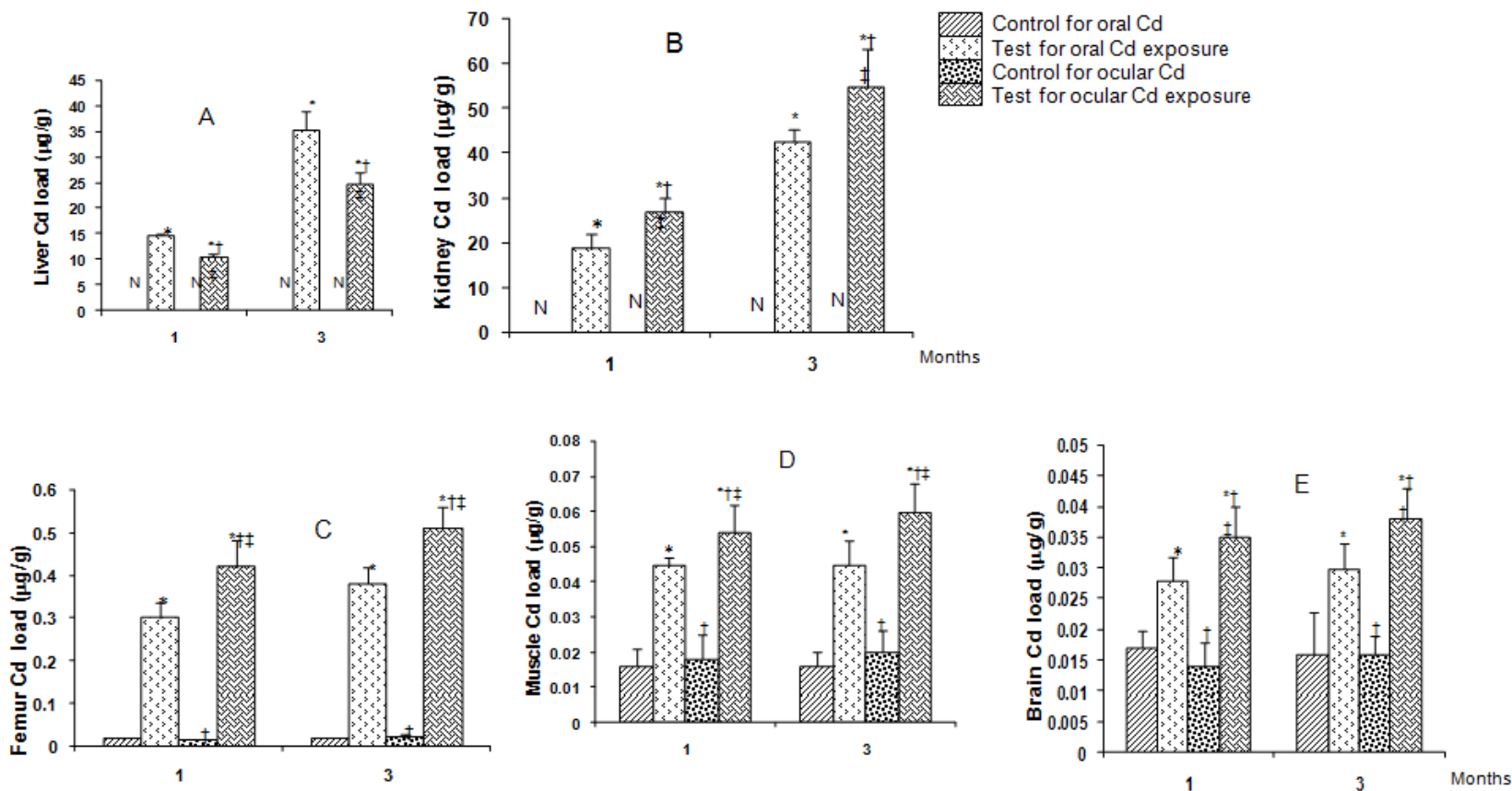


Figure 2. Concentration of cadmium in the tissues of rabbits after oral and ocular exposure. Values are means \pm SEM. $P < 0.05$ (Mann – Whitney U – test) compared to control of oral Cd exposure (*), test of oral Cd exposure (†) and control of ocular Cd exposure (‡) groups. Cd concentration in some control organs are denoted with N because they are negligible with respect to the test organs.

to uncoupling of ATP production in the mitochondria (Liu and Liun, 1990; Yoshioka et al., 1995). Thus, a compensatory increase in food consumption may be necessary in order to cope with the challenges of energy depletion.

The significantly lower body weight gain of rabbits after both mode of exposure to Cd (Figure 1A)

is in agreement with previous reports (Horiguchi et al., 1996; Asagba et al., 2004; Eriyamremu et al., 2005; Chater et al., 2009) and is an indication that the effect of Cd on weight gain may be independent of route of administration. The significantly lower body weight gain of rabbits exposed to oral Cd relative to those exposed to ocular Cd may not

be unconnected with the effect of oral Cd on nutrient digestion and availability as reported by Eriyamremu et al. (2005). This may be responsible for the significantly lower feed efficiency (Figure 1C) and the corresponding higher fecal output of these animals (Figure 1D).

It was observed that the kidney and the liver

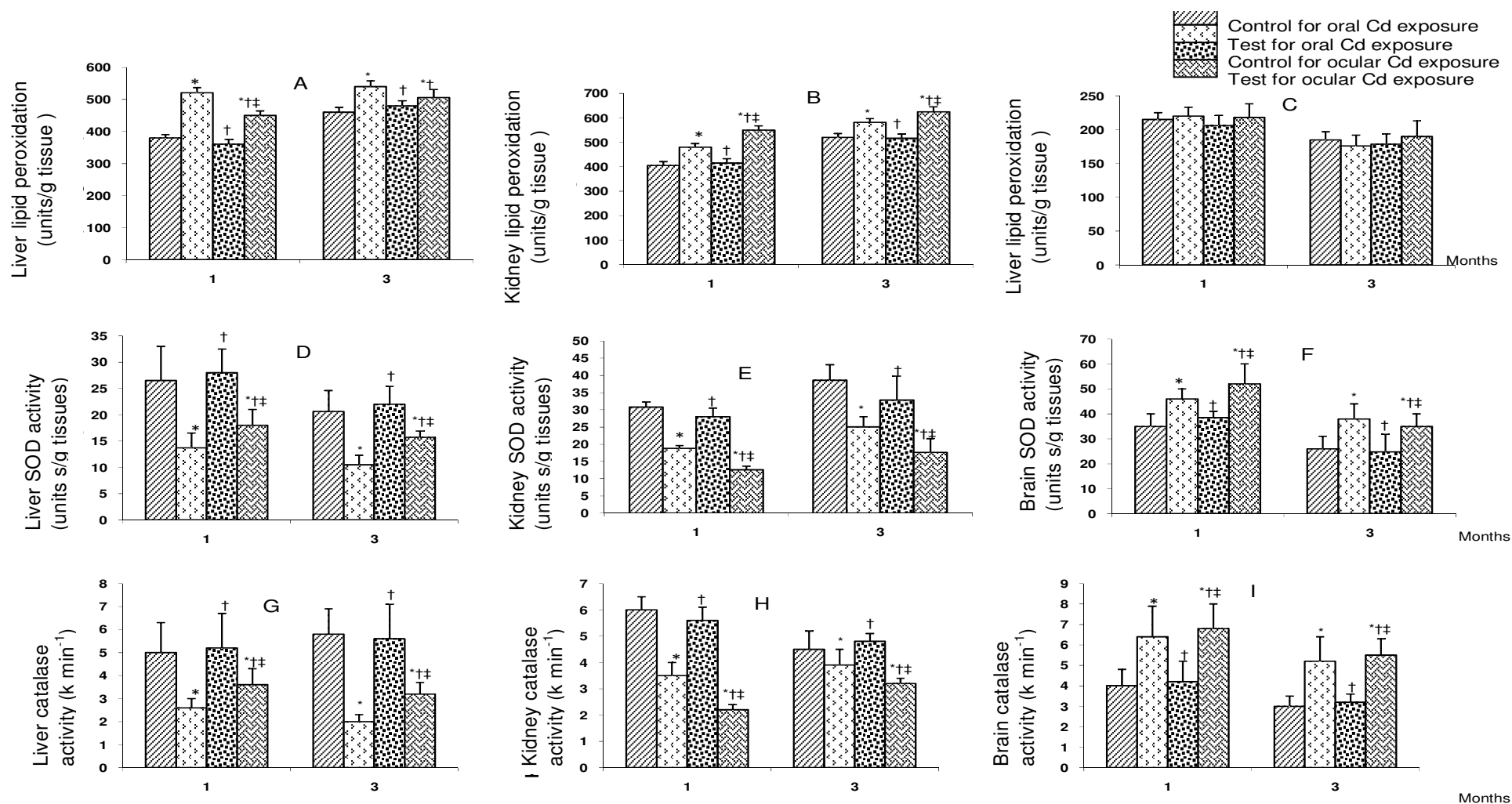


Figure 3. Effect of oral and ocular cadmium exposure on liver, kidney and brain lipid peroxidation and antioxidant enzymes activities. Values are means \pm SEM. $P < 0.05$ (Mann – Whitney U – test) compared to control of oral Cd exposure (*), test of oral Cd exposure (†) and control of ocular Cd exposure (‡) groups.

concentrated more Cd than the brain and femoral muscle (Figure 2). The relatively low level of Cd in the brain of the one and three

months exposed rabbits after both modes of exposure (Figure 2E) is consistent with the findings of a previous paper (Eriyamremu et al.,

2008b). So, despite the fact that Cd was administered through the eyes which have a close relationship with the optic nerve and then

brain, the metal accumulated more in the liver and kidney (Figures 2A and B) than in the brain (Figure 2E). This underscores the importance of the blood-brain barrier which has been reported to limit the uptake of Cd into the brain (Crowe and Morgan, 1997) unlike the liver and kidney which do not possess such a barrier mechanism.

The increased accumulation of Cd in the kidney and liver is consistent with previous reports which indicate that these two organs account for most of the body burden of Cd (WHO, 1992; Crowe and Morgan, 1997). The increased accumulation of Cd in these organs has been attributed to their ability to induce the synthesis of metallothionein (MT) (Klaverkamp et al., 1984; Timbrell, 2000). MT is a heavy metal binding protein which is believed to influence the uptake, distribution and toxicity of Cd (Wimmer et al., 2005; Klaassen et al., 2009). Besides, the liver and kidney are more exposed to foreign substances than other organs because they are the sites of metabolic activities and this may have also contributed to the increased concentration of Cd in these organs. The significantly higher concentration of Cd in the kidney, femoral muscle, femur and brain of rabbits after ocular exposure as compared to that of rabbits after oral exposure (Figure 2) may be due to the direct entry of the metal into the systemic circulation from the ocular tissue. Of considerable importance is the significantly higher amount of Cd in the liver after oral uptake than after ocular uptake in the one and three months Cd exposed rabbits (Figure 2A). Oral Cd enters into the body from the gastrointestinal tract by absorption into the portal blood system and pass via the portal vein to the liver. Thus after the gastrointestinal mucosa and blood, the liver is the next tissue to be exposed to oral Cd and as it is prior to dilution in the systemic circulation, this exposure will often be at a higher concentration than that of other tissues. Therefore, it can be hypothesized that the significantly lower amount of Cd in the liver after ocular uptake may probably be due to the omission of the portal blood system after ocular uptake of Cd. It is also noteworthy that irrespective of the route of exposure, the kidney (Figure 2B) accumulated the highest amount of Cd than the other organs. This may not be unconnected with the fact that the kidney as an excretory organ is the final destination of all the Cd from various tissues. The present finding on the higher uptake of Cd in the kidney as compared to other organs is in harmony with available reports in literature (Horiguchi et al., 1996; Baykov et al., 2003; Eriyamremu et al., 2008a).

The results of the present study have clearly demonstrated the ability of Cd to induce oxidative stress in rabbit tissues, as evidenced by increased lipid peroxidation and inhibition of enzymes required to prevent such damage after one and three months of Cd treatment (Figure 3). Accordingly, the depletion of SOD and CAT activities and increase in concentration of MDA after both modes of exposure were largely consistent with the level of Cd in the tissues. This finding is in agreement with

several reports demonstrating that Cd induces oxidative stress in tissues by increasing lipid peroxidation and by inhibiting antioxidant enzymes (Yalin et al., 2006; Hassan and Sahar, 2007; Chater et al., 2009; Renugadevi and Prabu, 2010). Several mechanisms have been postulated for the inhibition of antioxidant enzymes by Cd. Some of these mechanisms involve the displacement of essential metal cofactors from the active sites of the enzymes or the formation of covalent bonds by cadmium with sulphhydryl and other groups essential for the action of these enzymes (Timbrell, 2000; Casalino et al., 2002). It is noteworthy that there was no significant change in level of LPO in the liver of rabbits after ocular exposure to Cd for three months (Figure 3A) despite the inhibition of the antioxidant enzymes (Figure 3D and G). This finding is an indication that other antioxidant molecules may be involved in the protection of the liver from the effect of Cd induced reactive radicals. MT and glutathione are some of the antioxidant molecules that have been implicated in the adaptation of animals to Cd toxicity (Gupta et al., 1991; Klaassen et al., 2009). MT and glutathione bind Cd with the SH groups of their cysteine residues forming a complex which renders the metal non toxic. The metal is transported in this form to the kidney where it is finally excreted. Besides, the protection offered by MT and glutathione in Cd toxicity has also been attributed to the ability of these molecules to scavenge free radicals in cells (Gupta et al., 1991; Klaassen et al., 2009).

The significantly higher levels of SOD activity in the brain of rabbits after both mode of exposure to Cd (Figure 3F) underscores the sensitivity of the brain even to small amounts of Cd. SOD is inducible and the level of this enzyme will always increase with increasing need to protect against toxic oxidations (Aksnes and Njaa, 1981). Thus the significantly increased brain SOD and CAT activities (Figures 3F and I) may be partly responsible for maintaining oxidative damage in this organ at a level that is the same with that of the Cd-free control rabbits during both periods of exposure (Figure 3C). The similar SOD and CAT activity profiles in the brain of rabbits after both modes of Cd exposure (Figures 3F and I) is not surprising as the product of the action of SOD is the substrate for CAT. This finding is in agreement with previous reports which show that both enzymes which are involved in peroxide catabolism are not only functionally linked, but also occur in tandem (Bartkowiak and Bartkowiak, 1981; Halliwell, 1994; Asagba and Obi, 2000).

Plasma hepatic marker enzymes namely: L-AST, L-ALT and ALP were also measured in the present study as raised levels of these enzymes in the plasma are indications of liver damage (Annino and Giese, 1976). Thus the increased activity of these enzymes observed in the plasma of Cd exposed rabbits (Figure 4) is an indication of hepatic damage in these animals. The recent studies of Chater et al. (2009) and Renugadevi and Prabu (2010) also showed an increase in the activities of plasma L-ALT, L-AST and ALP as a result of

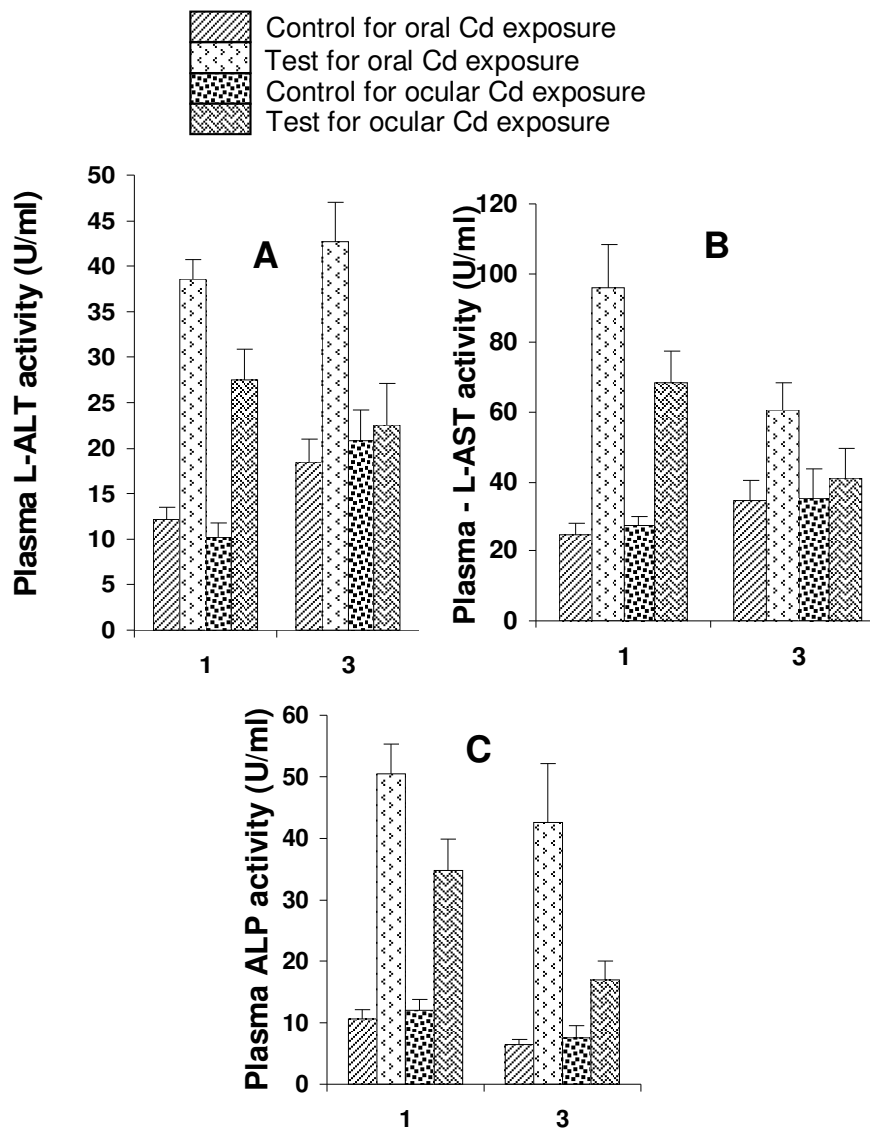


Figure 4. Effect of oral and ocular cadmium exposure on activities of L-ALT, L-AST and ALP in the plasma of rabbits. Values are means \pm SEM. $P < 0.05$ (Mann – Whitney U – test) compared to control of oral Cd exposure (*), test of oral Cd exposure (†) and control of ocular Cd exposure (‡) groups.

Cd exposure. The mechanism of liver damage in Cd exposure has been linked to a direct action of free Cd ions not bound with MT and also to reactive radical production which may lead to changes in functions and structure of many system and organ (Kowalczyk et al., 2002; Stohs et al., 2001). It follows therefore that, the increased level of membrane lipid peroxidation in the liver of these rabbits may account for the damage to this organ. The significantly lesser activities of the liver function enzymes in rabbits after ocular cadmium exposure for one month is an indication that exposure to Cd by this mode was less toxic to the liver than oral exposure to the metal in rabbits. This finding is consistent with the decreased accumulation of Cd in the liver of

rabbits ocularly exposed to the metal (Figure 3A). The lower level of Cd in the liver of these rabbits may have enabled them to recover from the toxic effect of the metal on the liver after three months of exposure as they had no significant change in plasma L-ALT and L-AST activities relative to control (Figure 4A), although the plasma ALP was elevated (Figure 4C). The lack of significant change in level of LPO in the liver of rabbits ocularly exposed to Cd for three months in relation to control (Figure 3A) lends further credence to the lack of liver damage in these rabbits.

In conclusion, this study has provided evidence for the accumulation of Cd in the tissues after exposure via the ocular route. However, most importantly, the study also

demonstrated that in relation to ocular exposure, oral exposure to Cd was more toxic to the liver. The differential effect on the liver is due to the differences in concentration of Cd in the liver after both modes of exposure to the metal.

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