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INFLUENCE OF DMSA AND EDTA ON LEAD MOBILIZATION AND REDISTRIBUTION IN ALBINO RATS

(With 7 Tables)

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تأثير الد مسا والاديتا على تحريك واعادة توزيع الرصاص فى الفئران البيضاء بعد تعرضها للرصاص

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فى هذه الدراسه تم استخدام سبعين من الفئران البيضاء قسمت الى ثلاث مجموعات (أ، ب، ج). أعطيت المجموعتان أ ، ب منها جرعات من الرصاص (خلات الرصاص) عن طريق ماء الشرب (٥٠، ١٠٠ جزء فى المليون) لمدة ثلاثة شهور وأستخدمت المجموعة الثالثة كضابط للتجربة. بعد ذلك قسمت كل من المجموعتين أ ، ب الى أربعة أقسام على النحو التالى (أ١ ، أ٢، أ٣، أ٤، ب١، ب٢، ب٣ ، ب٤). تم معاملة كل من المجموعات أ١، ب١ بمحلول فسيولوجى (٢سم/ كجم/ يوميا) كما تم معاملة المجموعات أ٢، ب٢ بالاديتا (١٠٠مجم/ كجم/ يوميا) وذلك عن طريق الحقن البريتونى لمدة أسبوع. بينما أعطيت كل من أ٣، ٣ب (٢٧مجم من الدايميركابتو سكسينيت DMSA لكل كجم من وزن الفأر مرتين يوميا عن طريق استخدام انبوبة اللى المعدى لمدة أسبوع أيضا) أما الاقسام أ٤ ، ب٤ فقد جرعت بخليط من DMSA , Ca Na_2 EDTA بنفس الجرعات السابقه و الطرق. وقد أظهرت الدراسه أهمية استخدام المركبين (Ca Na_2 EDTA, DMSA) معا كوسيله هامة فعالة مقارنة بتأثير كل منهم على حده فى تقليل نسبة الرصاص فى كل من الكبد والكلى والعظام كما أظهرت النتائج أن DMSA كان له تأثير اقوى فى تقليل نسبة الرصاص فى المخ كما أعطى أيضا نتائج طيبه مقارنة بالاديتا فى قياس بعد التغيرات البيوكميائيه وكذلك فى عدد كرات الدم البيضاء (Differential leucocytic count) مما يدفعنا للتوصيه باستخدام خليط المركبين فى علاج حالات التسمم المزمن بالرصاص.

SUMMARY

Seventy male albino rats were used. The rats were divided into three groups. The first, A and second B have been given 50 and 100 ppm lead, as lead acetate in drinking water respectively for three months. The third group, C was used as control. After that group (A and B) each was divided into four subgroups. These subgroups were given the recommended doses of either saline, mono calcium disodium ethylene diamine tetraacetic acid ($CaNa_2EDTA$), meso-2,3-dimercaptosuccinic acid (DMSA) or combination of $CaNa_2EDTA$ and DMSA for one week. The animals were sacrificed, blood, liver, kidney, brain and bone were taken for determination of lead, copper and zinc. Serum creatinine, enzymatic activities (S-AST, serum glutamic oxalacetic transaminase; S-ALT, serum glutamic pyruvic transaminase and S-ALP, serum alkaline phosphatase) and differential leucocytic count were estimated. The results indicated that: (I) The combination of CaNa2EDTA and DMSA was more efficient in reducing Pb concentration of liver, kidney and bone in comparison with each alone. However, the administration of DMSA alone was more effective than $CaNa_2EDTA$. (II) The brain Pb was depleted by DMSA more than by $CaNa_2EDTA$, while the combined chelation gave the same results as DMSA. (III) In the respect of differential leucocytic count, serum creatinine and the enzymatic activities, the DMSA gave the best results.

Keywords: *Lead toxicity - $CaNa_2EDTA$ - DMSA - liver - brain - kidney - bone.*

INTRODUCTION

The frequent cause of lead (Pb) poisoning in farm animals is grazing on high-lead plants, e.g. in the vicinity of lead smelting plants, or ingestion of toxic Pb compound, mainly dyes or substances used for their preparation, carelessly left in places to which animals have access. Cattle may be poisoned by licking used motor oil split on pastures, or rejected oil filters. Strips of land along busy highways may be contaminated with Pb pollutants produced by motor vehicles. After combustion, the tetraethyl Pb contained in petrol settles as Pb oxide or chloride on the vegetation by roadsides. Cases of Pb poisoning are most frequent

encountered in ruminants, followed by horses, poultry, dogs, cats and pigs (Bartik & Piskac, 1981).

The clinical signs due to lead poisoning were recorded by Clarke et al. (1981) in cattle and birds, Egan and O› Cuill, (1970) in sheep and horses, Zook et al. (1972) in dogs, by Scott (1963) in cats and by Bartik and Piskac (1981) in pigs.

Twenty years ago the therapeutic agent of choice to treat Pb poisoning in animals is sodium calcium edetate. The dose rate recommended for all species is 75 mg/kg daily. It should be administered by slow i.v. injection. In acute cases the treatment should be continued for three or four days. For the first 48 hours the daily dose should be divided and given at intervals of few hours. The use of $CaNa_2EDTA$ should be accompanied by supportive therapy. Barbiturates may be used to combat excitement or convulsions, cathartics or enemas for constipation and glucose-saline for anorexia and dehydration. Ascorbic acid (200 mg by mouth daily) was used with success in treatment of chronic Pb poisoning in lambs. In the absence of $CaNa_2EDTA$, recourse may be to the older remedy such as magnesium sulphate, calcium citrate and vitamin D should be used (Clarke et al., 1981).

The distribution of Pb in the environment is a major health hazard and intoxication with Pb may occur as pandemics in humans (Needleman, 1980). Pb intoxication is known to occur as a consequence of various forms of exposure. A more general environmental distribution of Pb, e.g., contamination of drinking water, food, or air Pb to chronic exposure (EPA, 1986). Pb intoxication can also occur due to a more short term exposure from specific sources, e.g., ingestion of leaded-paint chips or inhalation of Pb-contaminated dust (Atsdr, 1988).

Chelation for heavy metal intoxication has been practiced in various forms for approximately 40 year. The treatment by a combination of chelating agents has proven more effective than a single chelating agent (Jones, 1983; Andersen, 1987). The efficacy of $CaNa_2EDTA$ for the treatment of occupational Pb poisoning is well established (Chisolm, 1974; Piomelli et al., 1984). However, the source of the body Pb stores mobilized following $CaNa_2EDTA$ replication is a subject of dispute. Hammond and Co-authors (1967) investigated the changes in tissue Pb burden after intravenous $CaNa_2EDTA$ administration. These reports concluded that $CaNa_2EDTA$ mainly released Pb deposited in bones although a direct independent effect on soft tissues was postulated. Bone

depletion of Pb occurred during the period of $CaNa_2EDTA$ infusion, whereas soft tissue mobilization generally followed the infusion period. Castellino and Aloj (1965) indicated that $CaNa_2EDTA$ did not mobilize Pb from bone but from soft tissues following intravenous infusion. Cory-Slechta et al.(1987) recently supported the contention that the primary source of Pb mobilized by $CaNa_2EDTA$ is from bone with an additional contribution from kidneys. A provocative and most noteworthy finding of Cory-Slechta et al. (1987) was the observation that Pb mobilized by $CaNa_2EDTA$ from bone and kidneys may recirculate back to bone and brain during continued treatment, raising question of safety. Beyond the risk for increased brain Pb, $CaNa_2EDTA$ has several other disadvantages. Urinary losses of copper and zinc are significant during treatment (Flora and Tandon, 1990; Flora et al, 1990; Flora, 1991). Prolonged administration of $CaNa_2EDTA$ has also been associated with nephrotoxicity (Doolan et al., 1967; Tandon et al., 1986). On a molar basis, DMSA at a dose 1.5 time less than that of $CaNa_2EDTA$ has been shown to be more effective than $CaNa_2EDTA$ in mobilizing Pb from soft tissues in rats (Cory-Slechta et al., 1987; Cory-Slechta, 1988). It has recently been approved for use as an orally administered treatment for Pb toxicity (Angle, 1993). The chelating agents DMSA, a compound recently approved by the Food and Drug Administration for use in the USA in treatment of individuals with blood Pb levels above 45 microgram/dl and which is very useful in the treatment of childhood Pb intoxication (Graziano et al., 1992) and $CaNa_2EDTA$, which has been used to treat Pb intoxication in the clinic for about 40 years (Glotzer & Bauchner, 1992). The present study was designed to: (a) investigate the efficacy of $CaNa_2EDTA$ and DMSA either individually or in combination on mobilization and redistribution of lead in lead exposed-albino rats. (b) to study the effects of the chelating agents on creatinine and some enzymatic activities (ALT, AST & ALP) as well as the differential leucocytic count. These compounds were selected because of their actual or potential clinical interest.

MATERIALS and METHODS

Chemicals:

Lead acetate trihydrate (analytical grade) and $CaNa_2EDTA$ were purchased from Merck. DMSA was from Sigma. All other reagents used were of analytical grade.

Animals and treatment:

The present study was carried out using 70 male albino rat weighing 164 ± 4.3g. They were supplied by the experimental Animal Reseach Center of the faculty of Medicine, Assiut University. The rats were acclamitized two weeks before experimentation under standard laboratory conditions.These rats were divided into three groups 30 animals in each of the first and second group and only 10 in the third. First and second group (A & B), received Pb acetate in drinking water (50 ppm = 0.241 moles/litre and 100 ppm = 0.483 moles/litre respectively) for three months. Third group (C), received unleaded water also for three months. After three months exposure, groups A and B (each of them) were divided into four subgroups and treated as follows. Subgroup A1, n=3 and B1, n=3 (treated with 2 ml saline/kg. b.w. i.p. daily for one week. Subgroup A2, n=5 and B2, n=3 (treated with 100 mg $CaNa_2EDTA$ /kg. b.w. i.p. daily one time at 11 hr for one week. Subgroup A3, n=5 and B3, n=4 (treated with 27 mg DMSA /kg twice daily orally by stomach tube at 10 hr and 14 hr for one week. Subgroup A4, n=5 and B4, n=3 (treated with both $CaNa_2EDTA$, 100 mg/kg. b.w. i.p. and DMSA, 27 mg/kg. b.w. orally by stomach tube). All the animals were sacrificed and blood, liver, kidney, brain, femur and humorous were taken for Pb, copper and zinc determination.

The doses of the chelating agents were selected as recommended in the literature (Cory-Slechta et al., 1987; Cory-Slechta, 1988; Flora et al., 1995). Although one higher dose of both chelators was examined by (Cory-Slechta et al., 1987; Cory-Slechta, 1988), we selected the lower dose because of possible adverse effects from a higher dose of $CaNa_2EDTA$ in combination with DMSA. DMSA was given orally while $CaNa_2EDTA$ was administered intraperitoneally because (1) these are the recommended routes of administration for the two chelators and (2) no data about their possible interaction were available if given via the same route. All the chelating agents were prepared fresh before administration. DMSA solution was prepared by neutralization with sodium bicarbonate

before use. At the beginning of the treatment with chelating agents, lead acetate administration was stopped.

Biochemical analysis:

AST and ALT were estimated according to Reitman and Frankel (1957) and ALP was determined according to Wright et al., (1972). Serum creatinine level was measured using a diagnostic kits (Boehringer Mannheim, Germany) according to Husdan and Raport (1968).

Metals estimation:

Standard procedures were used to estimate lead, copper and zinc in blood, liver, kidney, brain, femur and humorous. All glassware, pipette tips and plastic ware were rinsed with 25 % HNO_3 to avoid metal contamination. (a) For tissue lead determination, 1 g wet tissue of brain, liver, kidney and 5 ml blood were used. Duplicate wet tissue samples were treated with concentrated nitric acid and perchloric acid (2:1), samples were brought to a constant volume and determination of tissue lead was carried out according to Yeager et al.(1971), using a Perkin Elmer Model 5000 atomic absorption spectrophotometer (AAS) . Bone (femur and humorous) was scraped of all adhering tissues and ashed in a muffle furnace at 450 C for 48 hr. One gram of this ash was dissolved in concentrated nitric acid and prepared for AAS analysis. (b) Whole tissue copper and zinc was measured by using AAS according to Parker et al. (1968).

Differential leucocytic count:

Differential leucocytic count was done after Coles (1986).

Statistical analysis:

The obtained data were statistically analysed according to Snedecor and Cochran (1974).

RESULTS

Exposure to 50 and 100 ppm Pb for three months produced a significant decrease in body weight gain from the 2 nd week (table 1). Also in this table showed obvious decrease in body weight gain in group A rather than in group B was recorded. The treatment with chelating agents resulted in increase body weight again (table 2). In table 2 all groups except B3 and B4 were significantly different from group C at the last week of the experiment. Also, only group A4 was significantly different from A1, while group B3 was significantly different from B1.

Table 1: Body weight gain of rats exposed to lead acetate (in grams).

Week number	Group c	Group A	Group B
Zero	164.0 ± 1.3	164.0 ± 1.3	164.0 ± 1.3
1	167.5 ± 2.0	160.1 ± 1.7^{c}	167.6 ± 2.2
2	171.0 ± 3.4	156.5 ± 1.8^{b,c}	166.6 ± 2.4
3	170.5 ± 2.1	146.8 ± 2.0^{b,c}	161.3 ± 2.1^{c}
4	171.5 ± 3.4	153.6 ± 2.0^{b,c}	160.8 ± 1.8^{c}
5	169.5 ± 2.1	156.3 ± 1.9^{b,c}	161.0 ± 1.9^{c}
6	171.5 ± 4.2	156.7 ± 1.1^{b,c}	165.3 ± 1.2
7	172.0 ± 2.2	155.4 ± 1.8^{b,c}	165.5 ± 2.6
8	172.8 ± 1.9	158.6 ± 1.3^{a,c}	163.0 ± 2.1^{c}
9	174.0 ± 2.5	161.2 ± 1.9^{c}	164.9 ± 1.9^{c}
10	174.8 ± 3.2	160.7 ± 2.2^{c}	160.6 ± 1.7^{c}
11	177.0 ± 3.0	161.2 ± 1.6^{c}	161.0 ± 1.6^{c}
12	177.8 ± 3.0	162.1 ± 1.7^{c}	166.6 ± 2.0^{c}
13	180.0 ± 2.8	162.7 ± 1.6^{c}	165.7 ± 3.3^{c}

a,b: Significantly different from group C at starting of the experiment at $p<0.05$ & $p<0.01$.

c: Significantly different with group C of each corresponding week at $p<0.05$.

- The obtained values are mean ± S.E.M.

Table 2: Influence of different chelating agents on body weight gain of albino rats previoussly exposed to lead. (in grams).

Type of group	Body weight after use of chelating agents
A2	163.0 ± 3.0^{b}
A3	165.2 ± 3.5^{a}
A4	170.0 ± 1.5^{a,c}
B2	169.3 ± 3.2^{a}
B3	182.0 ± 5.7^{d}
B4	167.3 ± 7.2

- Body weight gain of group C, A1 and B1 were 180 ± 2.8, 161.2 ± 1.5 and 163 ± 2.1 respectively.

a,b: means significantly different from group C at $P < 0.05$ & $p < 0.01$.

c: means significantly different from group A1 at $P < 0.05$.

a,b: means significantly different from group B1 at $P < 0.05$.

- The obtained values are mean ± S.E..

However, no appreciable changes in food and water intake were noticed especially in group C and group B through the time of experiment.

The effects of treatment on some serum biochemical variables are shown in table (3), AST, ALT, ALP and s-creatinine levels were shown in group A, B in comparison with group C. But group B showed more elevation in these previous variables than that induced in group A. After treatment of rats in group A with different chelating agents, AST and ALT in group A3 return back near to that in group C. On an another hand, ALP in group B2 was found to be similar in some extend to that in group C. Collectively treatment with chelating agents separately or in combination caused slight significant effects on these biochemical parameters.

The effect of DMSA and $CaNa_2EDTA$ treatment on Pb concentration of rats tissues is shown in table (4). DMSA was more effective chelating agent than $CaNa_2EDTA$ in decreasing blood, liver, kidney and brain Pb burden but $CaNa_2EDTA$ was more effective in depleting bone Pb. However, the chelating agents when given in combination form were significantly more effective in the removal of lead from blood, liver and kidneys.

Table 3: Some biochemical parameters in lead exposed and treated rats.

Group	S-GOT	S-GPT	S-ALP	S-Creatinine
C	4.11 ± 0.25	4.05 ± 0.28	6.52 ± 0.19	0.17 ± 0.01
A	5.18 ± 0.31^{a}	4.56 ± 0.15	6.87 ± 0.13	0.26 ± 0.02^{a}
B	6.01 ± 0.15^{b}	5.53 ± 0.10^{b}	7.10 ± 0.10^{a}	0.34 ± 0.01^{b}
A2	5.08 ± 0.17^{a}	5.03 ± 0.12^{a}	6.25 ± 0.14^{c}	0.22 ± 0.01^{a}
A3	4.69 ± 0.14	4.20 ± 0.17	7.61 ± 0.30^{a}	$0.32 \pm 0.01^{b,c}$
A4	5.05 ± 0.40^{a}	$6.14 \pm 0.11^{b,d}$	$7.83 \pm 0.19^{b,d}$	$0.39 \pm 0.02^{b,d}$
B2	5.71 ± 0.19^{b}	$6.43 \pm 0.22^{b,e}$	6.42 ± 0.11^{f}	0.37 ± 0.01^{b}
B3	5.53 ± 0.23^{b}	$6.56 \pm 0.30^{b,e}$	$7.98 \pm 0.16^{b,f}$	0.35 ± 0.02^{b}
B4	5.47 ± 0.21^{b}	$6.35 \pm 0.16^{b,e}$	7.15 ± 0.1^{a}	0.33 ± 0.02^{b}

- Values are mean ± S.E.M.
- Unites: s-GOT & s-GPT, nmol hydrozone formed/min/mg protein; s-ALp, µmol nitrophenol liberated/min/mg protein; s-creatinine, mg/dl.

a,b: Significantly different from group C at $p < 0.05$ and $p < 0.01$
c,d: Significantly different from group A at $p < 0.05$ and $p < 0.01$.
e,f: Significantly different from group B at $p < 0.05$ and $p < 0.01$.

Interestingly, there was a more pronounced decrease of Pb in the brain on combined treatment than the effect of DMSA alone. However, the decrease of lead content in both femur and humorus appears to be nearly similar as that resulted from $CaNa_2EDTA$ alone, and by so $CaNa_2EDTA$ was more effective than DMSA in mobilizing bone Pb burden (table 4).

Table 4: Effect of DMSA and CaNa2EDTA on lead concentration in blood and tissues.

Group	Blood	Liver	Kidney	Brain	Femur	Humorus
C	8± 0.5	2.4± 0.1	3.4 ± 0.1	0.22 ± 0.01	7.5± 0.2	7.6± 0.1
A	142± 4^{b}	14.6± 1.1^{b}	16.4± 0.4^{b}	0.91± 0.03^{b}	45.3± 1.9^{b}	43.3± 1.6^{b}
B	190± 5^{b}	26.7± 1.1^{b}	30.2± 0.8^{b}	1.52± 0.04^{b}	80.2± 1.1^{b}	81.1 $\pm 1.2^{b}$
A2	38± $2^{b,d}$	5.7± $0.1^{b,d}$	7.2± $0.1^{b,d}$	0.55± $0.02^{b,d}$	28.3± $1.9^{b,d}$	31.0 $\pm 1.6^{b,d}$
A3	54± $3^{b,d}$	9.6± $0.7^{b,c}$	13.4± $0.2^{b,d}$	0.85± 0.01^{b}	17.5± $1.0^{b,d}$	18.6± $1.1^{b,d}$
A4	31± $1^{b,d}$	3.7± $0.3^{a,d}$	5.3± $0.3^{b,d}$	0.47± $0.01^{b,d}$	10.1± $0.4^{b,d}$	10.6± $0.3^{b,d}$
B2	94± $3^{b,f}$	16.2± $1.1^{b,f}$	20.1± $0.2^{b,f}$	1.14± $0.02^{b,f}$	57.1± $0.9^{b,f}$	51.7± $0.7^{b,f}$
B3	121± $2^{b,f}$	19.1± $1.4^{b,f}$	25.0± $0.1^{b,f}$	1.43± 0.03^{b}	42.0± $1.0^{b,f}$	40.4± $1.0^{b,f}$
B4	78± $2^{b,f}$	12.8± $0.9^{b,f}$	17.6± $0.1^{b,f}$	1.05± $0.01^{b,f}$	40.6± $0.6^{b,f}$	37.1± $1.1^{b,f}$

a,b: Significantly different from group C $p < 0.05$ and $p < 0.01$
c,d: Significantly different from group A at $p<0.05$ and $p<0.01$.
e,f: Significantly different from group B at $p < 0.05$ and $p < 0.01$.
- The obtained values were mean ± S.E.M
- Unites: blood, µg/100 ml; soft tissues, µg/g wet tissue; femur and humorous, µg/g.

In table (5) blood zinc levels in group A4 and B4 were returned within normal, while in case of zinc level in the liver group B2 is appear the best. Zinc levels in kidneys in group A2 and B3 were approximately similar to that in group C. The best effect was shown in group A2 followed by A3 in case of zinc levels in the brain if compared with that in group C. Femur zinc levels showed an decrease in all groups treated with

chelating agents in comparison with group C or A and B, and this also similar to that occurred in the humorous. In table (6), in blood copper levels group A2, A4, B2 and B4 were nearly similar to that in group C although copper levels in group A3 and B3 markedly decreased if compared either with group C or A and B. In case of liver copper level, good results were obtained with B4 and A4 then followed by A2 and B2 while significant decrease was observed in B3 followed by A3. Kidney copper level in group B3 was significantly decreased when compared with that in group C, A and B. In all groups treated with chelating agents brain copper levels were near similar to that in group C. Copper levels in femur was significantly decreased in group B, A3 and B3. And in case of humorous group B, A4 and B3 showed an significant decrease when compared with group C.

Table 5: Effect of DMSA and CaNa2EDTA on zinc concentration in blood and tissues.

Group	Blood	Liver	Kidney	Brain	femur	Humorous
C	8.4± 0.4	26.5± 0.4	18.1± 0.5	11.2± 0.3	81.9± 1.6	80.8± 1.5
A	11.3± 0.5 b	29.8± 0.3 b	19.3± 0.5	14.3± 0.2 b	75.3± 1.2 a	76.2± 1.1
B	15.5± 0.3 b	31.2± 0.2 b	23.1± 0.2 b	18.4± 0.5 b	70.4± 1.1 b	69.1± 0.9 b
A2	15.1± 0.9 b,c	22.6± 0.5 b,d	17.5± 0.3 c	11.3± 0.4 d	57.8± 0.9 b,d	57.1± 0.8 b,d
A3	17.8± 0.2 b,d	19.2± 0.6 b,d	14.1± 0.4 b,d	10.8± 0.4 d	52.6± 0.7 b,d	51.9± 0.8 b,d
A4	6.9± 0.7 d	13.1± 0.4 b,d	10.6± 0.7 b,d	7.5± 0.3 b,d	51.5± 1.1 b,d	52.0± 1.0 b,d
B2	18.2± 0.6 b,e	25.6± 0.3 f	22.0± 0.4 b	16.8± 0.4 b	53.1± 1.2 b,f	52.3± 0.7 b,f
B3	20.3± 0.5 b,f	20.1± 0.3 b,f	17.9± 0.6 f	14.0± 0.2 b,f	50.2± 0.9 b,f	57.1± 1.2 b,f
B4	7.1± 0.2 f	16.8± 0.4 b,f	13.4 ± 0.5 b,f	12.9± 0.3 a,f	51.3± 1.1 b,f	55.2± 1.1 b,f

a,b: Significantly different from group C at $p < 0.05$ and $p < 0.01$.
c,d: Significantly different from group A at $p< 0.05$ and $p<0.01$.
e,f: Significantly different from group B at $p < 0.05$ and $p < 0.01$.
- The obtained values were mean ± S.E.M.
- Unites: blood, µg/100 ml; soft tissues, µg/g wet tissue; femur and humorous, µg/g.

The differential leucocytic count was disturbed in 50 ppm and 100 ppm lead exposed groups (table 7). The neutrophil cell percentage was significantly increased associated with decrease in the lymphocyte cell percentage. These percentages were improved and approached the control percentages in DMSA treated groups. In the groups which received the mixture of DMSA and $CaNa_2EDTA$, there were slight improvement especially in the 50 ppm lead exposed group. $CaNa_2EDTA$ treated groups showed no improvement in the differential leucocytic percentage. Monocytes showed significant increases in 100 ppm Pb acetate treated group and is highly significant in $CaNa_2EDTA$ treated group. While there is no changes in other groups. Percentage of eosinophil cells were decreased in the treated group either in significant or highly significant manner. There is no significant differences in the band cells and basophil cells from the control group.

Table 6: Effect of DMSA and CaNa2EDTA on copper concentration in blood and tissues.

Group	Blood	Liver	Kidney	Brain	femur	Humorus
C	3.9± 0.2	6.0± 0.1	8.1± 0.2	3.0± 0.1	39.1± 1.1	40.2± 1.6
A	3.5± 0.1	5.2± 0.1^{b}	6.9± 0.1^{a}	3.5± 0.2	36.1± 1.1	36.7± 1.1
B	3.3± 0.1	4.8± 0.1^{b}	6.1± 0.1^{b}	3.7± 0.1^{a}	32.1± 1.1^{b}	33.2± 1.0^{a}
A2	4.0± 0.1	6.7± $0.1^{b,d}$	6.1± $0.1^{b,c}$	2.7± 0.1^{c}	37.1± 1.2	36.1± 1.4
A3	2.7± 0.2^{a}	4.8± 0.1^{b}	5.0± $0.1^{b,d}$	2.8± 0.2	34.2± 1.3^{a}	35.1± 1.7
A4	4.1± 0.1^{c}	6.4± $0.1^{a,d}$	6.6± 0.1^{b}	2.7± 0.1^{c}	37.1± 1.2	34.2± 1.1^{a}
B2	4.0± 0.1^{e}	6.8± $0.1^{b,f}$	5.8± 0.1^{b}	2.5± 0.1^{f}	34.2± 1.0^{a}	34.0± 2.0
B3	2.3± $0.2^{b,f}$	4.2± $0.1^{b,e}$	4.3± $0.1^{b,f}$	3.0± 0.2	30.0 ± 1.2^{b}	31.3± 1.8^{a}
B4	4.0± 0.1^{e}	6.2± 0.1^{f}	6.0± 0.1^{b}	2.9± 0.1^{e}	35.3± 1.7	35.1 ± 2.1

a,b: Significantly different from group C at $p < 0.05$ and $p < 0.01$.
c,d: Significantly different from group A at $p < 0.05$ and $p < 0.01$.
e,f: Significantly different from group B at $p < 0.05$ and $p < 0.01$.
- The obtained values were mean ± S.E.M.
- Unites: blood, μ g/ml; soft tissues, μg/g wet tissue; femur and humorous, μg/g.

Table 7: Differential leucocytic count of rats exposed to lead and treated by chelating agents.

Group	Neutrophil	Band cell	lymphocyte	Monocyte	Basophil	Eosinophil
C	22.6 ± 0.6	2.2 ± 0.6	64.0 ± 1.1	8.8 ± 0.5	0.2 ± 0.1	2.2 ± 0.1
A	48.4 ± 2.2**	4.0 ± 0.8	37.4 ± 1.5	9.6 ± 0.6	zero	0.8 ± 0.3**
B	39.1 ± 1.0**	3.4 ± 0.5	45.6 ± 1.3**	10.6 ± 0.5*	0.1 ± 0.09	1.5 ± 0.1*
A2	28.7 ± 2.8	3.0 ± 0.5	56.0 ± 2.4*	10.2 ± 0.7	0.25 ± 0.2	1.7 ± 0.5
A3	52.2 ± 1.2**	3.2 ± 0.7	36.5 ± 1.7**	7.2 ± 0.7	zero	0.7 ± 0.2**
A4	19.6 ± 1.8	2.0 ± 0.4	67.3 ± 2.0	9.8 ± 1.5	0.16 ± 0.15	1.0 ± 0.2**
B2	20.3 ± 1.4	2.5 ± 0.5	66.8 ± 1.4	8.8 ± 0.7	0.16 ± 0.15	1.3 ± 0.3*
B3	26.6 ± 0.2**	2.6 ± 0.2	56.3 ± 1.0**	12.6± 0.2**	zero	1.6 ± 0.2
B4	31.7 ± 2.2**	5.0 ± 1.6	54.7 ± 1.7**	7.7 ± 0.4	zero	0.7 ± 0.2**

* : Significantly different from group C at $p < 0.05$.
** : Significantly different from group C at $p < 0.01$.
The obtained values were mean ± S.E.M.

DISCUSSION

Removal of lead from different intracellular deposits poses problems, as most of the chelating agents do not pass through the cellular membrane. The combination therapy involving at least two chelating agents with different modes of action might offer a solution to these problems (Dhawan et al., 1988). Chelation with $CaNa_2EDTA$ was widely adopted as a treatment for Pb poisoning after demonstration to relieve the clinical signs of intoxication and reverse some of the hematopoietic toxicity of Pb (Chisolm et al., 1976). DMSA was approved by the FDA in 1991 (FDA Medical Bulletin, 1991) for treatment of childhood Pb intoxication and increase urinary excretion of Pb in humans with high blood levels (Chisolm, 1990; Graziano et al., 1992).

The lower decrease of body weight gain in the group treated with 100 ppm lead acetate compared with that treated with 50 ppm may be due to the more enteropathic lesions in 50 ppm lead acetate treated group (this data will be published later in an another article by the same authors). The efficacy of DMSA in reducing soft tissue lead levels and of $CaNa_2EDTA$ in the mobilization of hard tissue lead prompted us to

examine the efficacy of DMSA alone and in combination with $CaNa_2EDTA$ in lead intoxication. It was anticipated that $CaNa_2EDTA$ would remove lead present in intracellular space, while DMSA would complex lead which had already passed into extracellular sites.

Our study shows that DMSA could significantly reduce the blood, liver, kidney and brain Pb levels after three months exposure to both 50 and 100 ppm Pb in the form of Pb acetate. The use of $CaNa_2EDTA$ resulted in deleading of Pb from liver and kidney in apart from the femur and humorous. Notwithstanding the significant mobilization of Pb by both DMSA and $CaNa_2EDTA$ individually, there was a significant additive effect as well. The reason is not understood, but may attributed to Pb mobilized from bone and excreted by the kidneys, which result in reduction of Pb redistribution to soft tissues (Cory-Slechta et al., 1987). Our study, further, demonstrated that DMSA is very effective in reducing not only blood, liver, brain and kidney Pb levels but also femur and humorous Pb contents was in agreement with Freidheim et al. (1976); Graziano et al. (1978); Flora et al. (1994) and Tandon et al. (1994). However, Cory-Slechta (1988) found that five daily injections of DMSA failed to produce any depletion of bone Pb. The redistribution of Pb mobilized from bone by $CaNa_2EDTA$ or non mobilization of Pb in response to DMSA treatment may be attributed to factors such as difference in dose and duration of Pb exposure contributing to the size of the Pb reservoir and in chelation ability of the chelators consequent to the changes in their pharmacokinetics under variable experimental conditions.

The most interesting results of DMSA in our study is its ability to reduce brain Pb more than $CaNa_2EDTA$ does. Our results are in full agreement with Chisolm (1990,1992), Flora et al. (1994, 1995), and Tandon et al. (1994). They concluded that DMSA is the most effective chelating agent examined for controlling Pb intoxication. The effective treatment regimen of Pb intoxication was probably due to (a) rapid depletion of Pb from bone and (b) subsequent mobilization of lead from soft tissue into urine and / or feces without redistribution or deposition.

A slight elevation of ALT activity due to DMSA administration alone which is in agreement with Graziano et al. (1985) who found a transient GPT elevation in two Pb poisoned patients also Flora et al. (1995) found that an elevation of GPT in Pb exposed rats. The elevation of s-creatinine and a marked decrease of blood zinc due to combined treatment with $CaNa_2EDTA$ and DMSA is in agreement with Flora et al.

(1995), which raises many questions regarding the safety of such regimens. The influence of zinc and copper supplementation during chelation therapy to reduce zinc and copper imbalance and promote Pb elimination from the body was investigated in rats poisoned with lead (Flora, 1991). The simultaneous supplementation of zinc and copper increased urinary Pb excretion by $CaNa_2EDTA$ compared to treatment with $CaNa_2EDTA$ alone. Combination therapy was effective in potentiating the depletion of blood and renal Pb by $CaNa_2EDTA$ and DMSA. So we are in agreement with his results that combination therapy ($CaNa_2EDTA$ and DMSA) was more effective in reducing hepatic Pb. From our results the copper and zinc status was changed which indirectly induce inhibition to blood delta-aminolevulinic acid dehydratase activity which result in increase in the urinary delta-aminolevulinic acid. Also dose-dependent effects of zinc supplementation in order to inhance the efficacy of $CaNa_2EDTA$ in lead intoxicated rats was investigated (Flora and Tandon, 1990; Flora et al., 1994). The results suggested that simultaneous supplementation with zinc at a lower concentration 10 mg/kg orally once during chelation treatment (Flora et al., 1994) and 25 mg/kg (Flora and Tandon, 1990) potentiates the mobilization of Pb from blood and soft tissues. However, at a higher zinc dose level 50 mg/kg once orally (Flora et al., 1994) it has deleterious and much reduced ability to deplete tissue Pb contents and restore altered Pb sensitive biochemical variables. So essential trace metal supplementation during chelation of Pb with oral zinc either alone or in combination with copper have been reported to be beneficial in animals given $CaNa_2EDTA$ therapy (Flora and Tandon, 1990 and Flora et al., 1994).

Differential leucocytic count indicated that DMSA was the best therapy followed by the mixture of DMSA and $CaNa_2EDTA$. While treatment with $CaNa_2EDTA$ had no effect in this respect, which was disagreed with Chisolm et al. (1976) who found that CaNa2EDTA reverse some of haematopoietic toxicity of lead.

Combined treatment with DMSA and CaNa2EDTA is more effective than treatment with one of them alone for the elimination of tissue lead. On the basis of the fact that $CaNa_2EDTA$ is potentially nephrotoxic, causes numerous loss of body zinc and is comparatively less effective than DMSA in mobilizing lead from all the examined tissues except bone, where in the former was most effective. The combined

chelation with $CaNa_2EDTA$ and DMSA is useful for optimum therapeutic uses with minimum adverse effects.

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