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EFFECT OF SUB CHRONIC EXPOSURE TO A MIXTURE OF WATER CONTAMINATING HEAVY METALS ON HAEMATOLOGICAL PARAMETERS AND FERTILITY OF FEMALE ALBINO RATS.

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ABSTRACT

In an environmental setup both humans and animals are exposed to mixture of heavy metals, especially through drinking water. Little is known about the interactions among various heavy metals during their co-exposure through drinking water and their net combined toxic implications in humans and animals. In current study, we examined the effect of sub chronic exposure via drinking water to a low dose (10 and 100 times mode concentration) mixture of three water contaminating heavy metals arsenic (As), cadmium (Cd) and lead (Pb) on haematological and reproductive parameters of female albino rats. The exposure to mixture for 60 days decreased body weight, weight of ovary and uterus and increased weight of liver and kidney. There was a significant decrease in red blood cell count, white blood cell count, and haemoglobin concentration. The clotting time increased significantly with concomitant decrease in platelet count. Fertility tests by pairing treated females with untreated males showed that mixture treated female were having an irregular oestrus cycle and a significant drop in fertility rate. Pups born to mixture treated animals showed loss in weight, high mortality rate and poor growth rate. The present study revealed that exposure to mixture of water contaminating heavy metals (As,Cd,Pb) caused changes in haematological parameters and affected reproductive performance of female rats at a low sub chronic dose.

KEY WORDS :Heavy metals (As,Cd,Pb), Haematology, Oestrous cycle, Fertility test.

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INTRODUCTION

Humans and animals are exposed concurrently to multiple metals via several routes especially through contamination of drinking water. Though several adverse health effects of metals have been known for long time, exposure to heavy metals is still increasing in some parts of the world, particularly in developing countries[1]. Most of the toxicological evaluations on water contaminating heavy metals involve assessment of exposure to single metal and at a dose much higher than World Health Organisation (WHO) suggested maximum permissible limit(MPL) of an individual metal in drinking water. Studies have revealed that sub chronic exposure to mixture of water contaminating metals at the MPL of individual metal can alter systemic physiology in both male and female rats. Though it is the fact that most of the environmental exposure to heavy metals is typically due to combination of multiple chemicals, little is known about interaction among various heavy metals during their co-exposure through drinking water and their net combined toxic implications in humans and animals[2]. Cadmium (Cd), lead (Pb) and arsenic (As) are all non-essential metals and are known to be toxic at very low levels. These non-essential metals can use transport and metabolic pathways that exists for biologically essential metals. The potential interactions between such non-essential and essential metals are possible and could be critical modifiers of metal toxicity[3]. Due to the multiplicity of interactions and combinations of metals, systemic and complete listing of each possible chemical interactions and combination for real world mixtures is practically impossible. In metal mixture toxicity studies, this problem is overcome by treating mixture as a single compound and testing it as a whole[4].Based on the criteria of frequency of occurrence in the environment, toxicity and potential exposure to humans; arsenic, lead and cadmium are considered as the most toxic substances. Survey of literature reveals that ground water contamination with these heavy metals are widespread in India[5]. There is very little information on adverse health effect associated with low level combined exposure to these heavy metals[6]. Therefore, in the current study, a mixture of these most frequently occurring heavy metals with a relative concentration similar to that detected in the groundwater sources of India was designed and the toxic potential of the whole mixture, containing arsenic, cadmium and lead was tested as a single compound to evaluate the net combined effects of the components in the mixture.

MATERIALS AND METHODS

Selection of Metals, Mixture Formulation

Metals were selected primarily on the basis of frequency of their occurrence and contamination level in drinking water. The most frequently occurring concentration of each metal was derived from the reported concentration and considered as the baseline dose[7]. The baseline dose (1X) for each chemical was adjusted to give the representative elemental concentration and then two more concentrations(10X and 100X) were incorporated for dose selection because of probable interspecies and inter individual variations as per the standard protocol. The relative concentrations of metals in mixture at different dose level are as follows:

Dose Level	Sodium arsenate (ppm)	Cadmium chloride (ppm)	Lead acetate (ppm)
Base Line Dose (1X)	0.380	0.098	0.220
10 Fold Base Line Dose (10X)	3.80	0.98	2.20
100 Fold Base Line Dose (100X)	38.0	9.8	22.0

Animals

Female rats (75-100gms) after acclimatization in the Institutional animal house were utilized for the study. Animals were given food and water ad libitum. They were kept in controlled condition of temperature (25± 2°C) and normal day/night schedule (12L: 12D).

Study design

The animals were divided in four groups of eighteen rats in each group. One of the groups of rats was maintained as control without any treatment (Control Group). The other rats were divided in to three treatment groups and were given mixture of metal in different concentration for 60 consecutive days:

i) Treatment Group I (1X) ;
ii) Treatment Group II (10X) and,
iii) Treatment Group III (100X).

The entire study protocol is approved by the institutional animal ethical committee and utmost care was taken during the time of sacrifice according to Indian Council of Medical Research (ICMR) guide lines.

Body Weight and Organ Weight

The weight taken on the 1st day was initial body weight and weight taken on day 61st before autopsy was considered as final body weight. The liver, kidney, heart, spleen uterus and ovaries 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.

Haematological study

The fresh blood was collected into heparinised test tubes immediately after sacrifice for haematological studies. The whole blood was used for estimation of haemoglobin concentration, Red blood cells(RBC), White blood cells(WBC), platelet count and determination of bleeding time by using standard techniques.

Study of Oestrous Cycle

State of oestrous cycle of each animal was determined by taking vaginal smears daily between 10.30A.M to 11.30 A.M during whole study period. In order to take vaginal smears, the vagina was washed with physiological saline (0.9%) by injecting a drop of solution with a dropper. The vaginal smears were examined immediately under the microscope while still wet and the cellular components were judged to determine the various stages of oestrous cycle[8] .

Fertility Test

Fertility test was conducted by cohabitating treated females with normal males in the ratio of 1:1. The female rats were then cheeked for presence of vaginal plugs or spermatozoa in the vaginal orifice, as evidence of mating. After successful mating, the male were removed from the cage. The day when vaginal plugs or spermatozoa were observed was considered as gestation day (GD0). To assess the implantation loss, some of the animals in each group were sacrificed on GD6 to observe the number of corporalutea and pre implantation sites. The remaining animals were sacrificed on GD18 to assess the post implantation loss, status of embryo (normal or dead) and the weight of embryos. The pre implantation and post implantation loss are assessed as follows:

Pre implantation loss (%)= (No. of corporalutea- No. of implantation sites) / No. of corporalutea X 100

Post implantation loss(%) = (No. of implantation- No. of live foetus) / No. of implantation X 100

The animals that completed gestation period, the parameters of birth rate, litter size, morphological alterations, and survival rate of pups were recorded[9].

Data analysis

Data were analysed by using analysed by using statistical software StatPac for Windows, version 11.0. Mean and SE values for each parameters in different groups were calculated. A post hoc “t” test was done to compare the differences between means. Significance was set at $p<0.05$.

RESULTS

The results of the study revealed that there was a significant decrease in percentage gain of body weight in rats treated with a mixture of cadmium, lead and arsenic at a dose of 100 times of baseline dose (Table I). The treatment resulted in significant increase in weight of kidney and liver with decrease in weight of ovary and uterus in treated female rats. Weight of other organs like heart and spleen did not very significantly (Table II). There was a significant decrease in haemoglobin concentration and total count of erythrocytes and leucocytes. Platelet count also decreased with a significant increase in clotting time in female rats treated with 100X concentration of mixture of lead, cadmium, and arsenic (TableIII).Study of oestrous cycle revealed treated female rats had prolong-diestrous with significant reduction of proestrus and oestrus phase of the cycle (Table IV).Treatment of female rats with the mixture of metals resulted into significant pre and post implantation loss in comparison to the control animals (TableV).There was a significant decrease in number of pups born to the treated female and 70% of the pups died within 15 days after parturition (Table VI).

Table I

Effect of treatment with Mixture of Metals on Body weight gain Of the animal ; Values are in Mean ± SE

Animal (6)	Initial body weight (g)	Final body weight (g)	Body weight gain (%)
Control	175±11	282±12	100
Treatment Group I	167±9	251±8	83
Treatment Group II	183±10	267±9	78
Treatment Group III	178±9	248±9	64 *

Number in parenthesis indicates no. of animal in each group. * p< 0.05

Table II

Effect of treatment with Mixture of Metals on weight of vital and reproductive organs of the animal; Values are in Mean ± SE.

Animal (6)	Liver (g/100gbw)	Kidney (g/100gbw)	Heart (g/100gbw)	Spleen (g/100gbw)	Ovary (g/100gbw)	Uterus (g/100gbw)
Control	3.72±1.00	0.40±0.08	0.52±0.08	0.38±0.13	0.40 ± 0.16	4.29 ± 0.73
Treatment Group I	3.12±0.84	0.45±0.13	0.40±0.06	0.42±0.09	0.47 ± 0.18	3.78 ± 0.90
Treatment Group II	3.34±0.92	0.56±0.14	0.68±0.10	0.49±0.11	0.34 ± 0.12	3.42 ± 0.97
Treatment Group III	4.90±0.40*	0.92±0.16*	0.96±0.01*	0.47±0.09	0.31± 0.12*	2.68± 0.63*

Number in parenthesis indicates no. of animal in each group. * p< 0.05

Table III

Effect of treatment with Mixture of Metals on haematological parameters of the animal ; Values are in Mean ± SE.

Animal (6)	Hb (gm/dl)	RBC Count (10^6/µl)	WBC Count (10^3/µl)	Platelet Count (10^3/µl)	Clotting Time (min)
Control	14.56±0.72	6.25±0.29	6.12±0.14	812.87±51.20	4.49±0.72
Treatment Group I	15.12±1.10	5.25±0.35	6.00±0.30	723.41±37.54	5.23±0.66
Treatment Group II	12.95±0.76	5.06±0.30	5.70±0.35	686.66±57.15	5.98±0.63
Treatment Group III	10.00±0.82*	4.20±0.40*	5.65±0.28*	526.33±53.61*	7.21±0.34*

Number in parenthesis indicates no. of animal in each group. * p< 0.05

Table IV
Effect of treatment with Mixture of Metals on oestrous cycle of the animal; Values are in Mean ± SE.

Animal (18)	Proestrus (% cycle)	Oestrous (% cycle)	Metestrus (% cycle)	Diestrus (% cycle)
Control	13.86±1.02	28.76±1.41	16.78±1.36	41.67±2.18
Treatment Group I	12.99±1.42	26.00±1.28	17.00±1.17	43.98±1.76
Treatment Group II	12.37±1.06	26.35±1.32	17.48±1.98	43.16±1.34
Treatment Group III	8.98±0.98*	24.67±1.12*	19.64±1.42*	48.36±1.54*

Number in parenthesis indicates no. of animal in each group. * p< 0.05

Table V
Effect of treatment with Mixture of Metals on implantation of the animal; Values are in Mean ± SE.

Animal (6)	No. of Corpora lutea	No. of implantation	Pre implantation loss (%)	No. of live foetus	Post implantation loss (%)
Control	12. 61 ± 0.91	11.35 ±0.86	20	9.67 ±0.75	15
Treatment Group I	12.42 ±0.95	10.64± 0.88	26	8.29 ±0.92	18
Treatment Group II	11.71± 0.98	10.83± 0.74	30	9.35 ±0.72	20
Treatment Group III	10.65± 0.53	8.21 ±0.36	70*	6.56 ±0.76	80*

Number in parenthesis indicates no. of animal in each group. * p< 0.05

Table VI
Effect of treatment with Mixture of Metals on survival rate of foetus of the animal;Values are in Mean ± SE.

Animal (6)	No. of pups	Survival at birth time	Survival after 15 days	Survival after 30 days	Survival after 45 days	Survival after 60 days
Control	8.92±0.68	100%	100%	100%	100%	100%
Treatment Group I	9.76±0.64	100%	100%	100%	100%	100%
Treatment Group II	8.16±0.94	100%	100%	100%	100%	100%
Treatment Group III	6.24±0.84	100%	30%*	30%*	30%*	30%*

Numbers in parenthesis indicate no. of animal in each group. * p< 0.05.

DISCUSSION

The purpose of the present study was to evaluate the effect of combined exposure to water contaminating heavy metal Pb, Cd and As on haematological and reproductive parameters in female albino rats. The present study revealed that low dose exposure to the mixture of three water contaminating heavy metals lead, cadmium and arsenic caused a dose dependent toxicity in female rats. At a relatively high exposure level of 100 times to the base line dose (100X) for 60 consecutive days, the toxicity was manifested as decrease in percentage gain in body weight with simultaneous decrease in food efficiency and water consumption. Decreased gain in the body weight in exposed animals relative to the control indicated the growth retarding effect of the mixture of these three heavy metals on female albino rats. Reduction in food and water intake might contributed to decrease in body weight in exposed animals[10]. Several studies have reported concurrent administration of Pb, Cd, and As in male rats reduced weight gain as a consequence of decreased food

utilization[11,12]. Also the present study showed that weight of liver and kidney were increased after the exposure period. Jadav et.al.observed similar effect of sub chronic exposure to eight water contaminating heavy metals including Pb, Cd and As[2]. The increase in the weight of liver and kidney, the organs predominantly associated with biotransformation of xenobiotics, in exposed animals might be indicative of body's adaptive mechanism to combat systemic toxicity. The obtained results are in agreement with findings of several other studies that dealt with toxic effects of heavy metals [13,14]. The observations strongly suggest that both liver and kidney are the primary target organs, posing a risk of hepatic and nephrotic damage to a chronic exposure of mixture of heavy metals through drinking water. These findings confirmed the previous observations that both liver and kidney are the primary target of heavy metal toxicity[15].

From our observation, it can be seen that treatment with mixture of Pb, Cd and As induces anaemia type condition with decrease in RBC count and haemoglobin concentration in female rats after 60 consecutive days of treatment. This findings are in agreement with recent findings of Nicolin et.al., which shows that the main effect of Pb and Cd intoxication were decreased erythrocyte, haemoglobin and haematocrit[16]. On the other hand arsenic is often taken up by RBCs and WBCs upon absorption, leading to haematological changes such as macrocytic anaemia, elevated eosinophil and basophilic stippling of RBCs[17]. In our study, we also observed a decrease in platelet count with concomitant increase in bleeding time in treated animals. Similar observations in rats are reported by Tikare et. al. with exposure to heavy metals like Nickle II and Chromium VI[18]. Study on oestrous cycle revealed that sub chronic treatment with the mixture of heavy metals Pb, Cd and As caused prolongation of diestrous with decrease in proestrous and oestrous phase in the reproductive cycle of treated female rats Similar prolongation of disastrous with reduction of oestrous and proestrous are observed in rats by various investigators after treatment with heavy metals[8,19,20]. The findings on implantation showed a significant pre implantation and post implantation loss in treated animals with decrease in the number of live foetus. Survival study of animals born to the treated female showed only 30% animals survived after 15 days of birth. Dhir observed similar effects on progenesis of pregnant female rats exposed to cancer causing heavy metal ions Cu^{2+}, Cd^{2+} and Pb^{2+}[21].

CONCLUSION

In conclusion, the dose related decrease in body weight gain, feed efficiency and increase in weight of metabolically active tissue liver and kidney revealed a general toxic effect of mixture of water polluting heavy metals lead, arsenic and cadmium at 100 times their baseline concentration in water. Decrease in weight of reproductive organs along with disturbances in oestrous cycle, decrease in number of corpora lutea of pregnancy, number of implantation sites and number of pups in female rats suggested that the metal might have affected the fertility of female rats by interfering with ovarian steriodogenesis. Decrease in number of corpealutea is due to hormonal imbalance at any stage in hypothalamohypophyseal ovarian axis or by desensitizing the ovary to gonadotropin. However, further investigation is needed to understand the real mechanism of action of combined exposure to a mixture of lead (Pb) , cadmium (Cd) and arsenic (As) in female reproductive system.

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