Bull Environ Contam Toxicol (2007) 79:273–277
DOI 10.1007/s00128-007-9225-4

Impact of Distillery Soil Leachate on Haematology of Swiss Albino Mice (*Mus musculus*)

Subhasini Sharma · Arti Sharma · Pawan Kumar Singh · Pratima Soni · Shweta Sharma · Pradeep Sharma · K. P. Sharma

Received: 5 October 2006 / Accepted: 15 June 2007 / Published online: 4 August 2007

Abstract This study reports significant alterations in various haematological parameters such as red and white blood corpuscles counts, haemoglobin content and packed cell volume in adult Swiss albino mice orally administered with diluted distillery soil leachate (5%–20%) for 30 days. Soil leachate also affected red blood cell morphology (poikilocytosis). The haematology of exposed mice improved in the reversal groups. Present study infers contamination potential of distillery soil leachate in the groundwater.

Keywords Distillery soil leachate · Haematology · Swiss albino mice

Distillery industry is one of the major chemical industries. Today there are about 295 distilleries in India with an installed capacity of 3,198 million L of alcohol production. About 12–15 L of spent wash is produced after 1 L of alcohol production (Bhaduri 1980). The wastewater of distillery known as spent wash, is dark brown in colour (due to melanoidins), acidic in nature (pH 3.94–4.30) (Banerjee et al. 2004), bioremediation resistant (Khanna et al. 2003), loaded with pressed pulp molasses, vinasses, organochlorines, organophosphates, herbicides, fungicides (Lopez et al. 2003), organic and inorganic salts as well as compounds like phenols, lignin, oil and greases, resulting in high electrical conductivity (EC) (30–45 ds/m). The values of biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolve solids (TDS) and biogenic salts (P and N) are several folds greater than the discharge limits as prescribed by Central Pollution Control Board (CPCB), New Delhi (1989). It also contains heavy metals such as iron, copper, zinc and chromium (Dubey 1993; Pathade 1999). The unpleasant odour due to the presence of skatole, indole and other sulphur compounds, which are not effectively decomposed by yeast or methanogenic bacteria during distillation, is also an issue of public concern. Disposal of this recalcitrant effluent on land effects soil fertility through leaching. It also results in accumulation of salts in soil making it saline (Devarajan and Oblisami 1995) and decrease its alkalinity and manganese availability thus makes soil unfit for cultivation. Such soil has been referred to as distillery soil here after in the text.

Distillery wastewater adversely affects the whole ecosystem of surrounding area. Several toxicological studies of distillery wastewaters were made on plants, while a very few studies were available on animals. Kumar and Gopal (2001) reported hematological alterations in *Channa punctatus*, exposed to distillery effluents. However, the reports on mammals are very scanty. Hence this work was undertaken to study the toxic effects of distillery soil leachate on hematological parameters of mice. It is expected that the parameters chosen are of clinical interest and will help in knowing about the deleterious effects of distillery soil leachate on animals and human health.

Materials and Methods

Distillery soil collected from the crop field near Behrod, Alwar district (Rajasthan, India) was dried in the sun. It was

S. Sharma · A. Sharma · P. Soni · S. Sharma
Department of Zoology, University of Rajasthan, Jaipur, India

P. K. Singh · P. Sharma · K. P. Sharma
Department of Botany, University of Rajasthan, Jaipur, India

S. Sharma (✉)
C-141A, Mahaveer Marg Malviya Nagar, Jaipur 302017
Rajasthan, India
e-mail: subhasini_sharma@yahoo.com

ground and passed through stainless steel sieve of 2 mm mesh size. Soil leachate prepared by stirring it in distilled water (1:1) for about 24 h, and allowing soil suspension to stand still for almost 4 h in a measuring cylinder. Clear supernatant was pumped out and filtered by passing through Whatman filter paper. Now prepared soil leachate was considered 100%, thereafter different concentrations viz. 5%, 10% and 20% were made by adding distilled water, on the basis of LD50 value calculated by using the COMPAQ personal computer BASIC version 1.13. Healthy mature (age, 45–50 days; weight, 35–45 g) Swiss albino mice (*Mus musculus*) from an inbred colony were acclimatised for 10 days prior to experiment. Three to four mice were kept in polypropylene cages at 25 ± 3°C, 40%–60% relative humidity and 12 h alternate light: dark cycle as per guidelines of the Institutional Ethical Committee. They were fed on mice feed from Hindustan Lever Ltd, India and potable water was provided ad libitum. These animals were divided into five groups having ten animals each.

Group I: Standard feed + potable water
Group II: Standard feed + 5% distillery leachate (15 days)
Group III: Standard feed + 10% distillery leachate (15 days)
Group IV: Standard feed + 20% distillery leachate (15 days)
Group V: Standard feed + 5% distillery leachate (30 days)

At the end of the experiment six animals of each group were anaesthetised by chloroform. Their blood was collected directly from cardiac puncture by sterilised disposable syringe and stored in vials having anticoagulant (EDTA). Thereafter, the remaining four animals of each group were maintained on standard feed and potable water to examine their recovery in control conditions, as for group I. They were sacrificed after 45 days as mentioned above. All haematological parameters viz., haemoglobin concentration (Hb), red blood corpuscle (RBC) count, white blood corpuscle (WBC) count, packed cell volume (PCV) with their indices mean corpuscle volume (MCV), mean corpuscle haemoglobin (MCH), mean corpuscle haemoglobin concentration (MCHC) were estimated by standard methods as prescribed by Dacie and Lewis (1977). To calculate the % abnormality among RBCs, 20 microscopic fields ($10_x \times 100_x$) were observed using an oil immersion. Statistical analysis of the data was carried out using SYSTAT computer program version 5.0.

Results and Discussion

The results of present investigation indicate profound changes in hematological profile of the test animals treated with distillery leachate. In comparison to control animals, RBC count (31%–33%), Haemoglobin concentration (24%–28%), PCV (30%) decreased significantly in leachate treatments, whereas an opposite trend was observed for MCV (21%–26%) and MCH (27%) (Table 1). Interestingly these changes were independent of leachate concentrations as evident by little difference in their observed values at various concentrations. The leachate exposure, however, had little adverse effect on WBC count and MCHC, since they were almost similar to control animals, with the exception of a pronounced increase (105%) in WBC count in 10% concentration. In the reversal groups, the values of hematological parameters increased (3%–69%) in comparison to treated mice, being maximum for WBC (69%) at 5% concentration after 30 days. However, increase was little lower (3%–15%) at 20% concentration, suggesting slow recovery in comparison to other treated animals (Table 1).

Distillery effluent exposure adversely affected haematology of Swiss albino mice, as noted in the present study. As described earlier, the major toxic pollutant in distillery effluent are phenols and heavy metals. Phenols are known to oxidise haemoglobin (Bukowska and Kowalska 2003) and provoke haemolysis of the cell (Duchnowicz et al. 2002). Heavy metal exposure affected haematology almost similarly (Reddy at el. 2003; Sateesh et al. 1999). Distillery soil leachate possibly suppressed haemopoietic system, which decreased RBC count, WBC count, Hb concentration and PCV. Further reduction in MCHC indicated poor haemoglobin carrying capacity of erythrocytes, which consequently decreased its level (Lynch et al. 1969; Oda et al. 1980; Eaton and Klaassen 1998). Decreased PCV is an indicator of effect of stress on animal health and O_2 carrying capacity of blood (Larsson et al. 1985). A decreased PCV which is accompanied with a fall in RBC count may be correlated with the degree of anemia. White blood cells are a part of body's defense immune system. Toxic chemicals of leachate combine with neutrophils to form complexes, which stimulate the production of antibodies that decreased WBC count due to an inhibitory mechanism (Kou et al. 1997). While an exceptional increase in its count (105%) in 20% treatment may be ascribed to metabolic disturbances (Guilthermino et al. 1998).

Red cell indicators like MCV, MCH and MCHC are dependent on the red blood corpuscle count, haemoglobin concentration and packed cell volume. Many investigators, e.g. Mathur et al. (2003); Chandravathy et al. (1996); Ghai (1993); Blair et al. (1990) observed an increase in MCV and MCH in albino rats/mice similar to present study. Increase in MCV suggests an intensified compensating activity of the haemopoietic system, which may be in response to haemolytic action of the toxicants. Increased

Table 1 Effect of distillery soil leachate on haematological parameters in albino mice after sub-chronic and reversal treatment

Days	Conc (%)	Treatment	Parameters						
			RBC ($\times 10^6$/mm^3)	WBC (mm^{-3})	Hb (g%)	PCV (%)	MCV (fl)	MCHV (pg)	MCHC (%)
15	5	Subchronic	6.1 ± 0.5* (−31%)	3783.3 ± 379.7^{NS} (−3%)	9.1 ± 0.7** (−28%)	39.6 ± 5.4^{NS} (−20%)	64.9 ± 6.1^{NS} (+14%)	15.2 ± 1.2^{NS} **(+6%)**	23.6 ± 2.2^{NS} (−7%)
		Reversal	11.1 ± 1.0^{NS} (+60%)	5468.5 ± 508.0^{NS} (+26%)	12.7 ± 0.9^{NS} (+31%)	55.4 ± 3.3^{NS} (+21%)	55.8 ± 2.0^{NS} (−15%)	12.8 ± 0.6^{NS} (−8%)	21.8 ± 1.4^{NS} (+3%)
	10	Subchronic	7.0 ± 0.6^{NS} (−21%)	3741.7 ± 994.5^{NS} (−4%)	11.4 ± 0.8^{NS} (−10%)	49.8 ± 4.1^{NS} (0%)	71.8 ± 2.8* **(+26%)**	16.4 ± 0.3^{NS} (+14%)	22.9 ± 1.1^{NS} (−10%)
		Reversal	7.4 ± 0.4^{NS} (+6%)	6014.0 ± 114.3^{NS} (+39%)	10.0 ± 2.0^{NS} **(+3%)**	53.1 ± 4.0^{NS} (+16%)	74.6 ± 1.5^{NS} (+13%)	14.9 ± 0.5^{NS} (+7%)	23.0 ± 16.3^{NS} **(+9%)**
	20	Subchronic	5.9 ± 0.8* **(−33%)**	7991.7 ± 227.4^{NS} **(+105%)**	9.6 ± 0.8* (−24%)	34.8 ± 3.2* (−30%)	59.4 ± 3.2^{NS} **(+5%)**	16.4 ± 0.9^{NS} (+14%)	27.7 ± 1.2^{NS} (+9%)
		Reversal	6.7 ± 0.8^{NS} (−4%)	4712.5 ± 1341.5^{NS} (+9%)	10.7 ± 1.7^{NS} (+10%)	48.8 ± 4.8^{NS} (+7%)	73.5 ± 1.6^{NS} (+12%)	16.0 ± 0.6^{NS} **(+15%)**	21.8 ± 1.3^{NS} **(+3%)**
30	5	Subchronic	6.7 ± 0.3* (−23%)	3641.7 ± 773.5^{NS} (−7%)	12.3 ± 0.7^{NS} **(−2%)**	45.8 ± 2.1^{NS} (−8%)	68.6 ± 5.6* (+21%)	18.3 ± 1.1* **(+27%)**	26.8 ± 1.4^{NS} **(+5%)**
		Reversal	7.8 ± 0.4^{NS} (+12%)	7350.0 ± 1028.1^{NS} **(+69%)**	12.5 ± 0.5^{NS} (+29%)	57.4 ± 3.0^{NS} (+25%)	74.0 ± 7.8^{NS} (+12%)	16.1 ± 0.2^{NS} (+15%)	21.9 ± 2.0^{NS} (+4%)
Control		Subchronic	8.8 ± 0.5	3900.0 ± 513.8	12.6 ± 0.2	49.8 ± 3.4	56.8 ± 2.9	14.4 ± 0.7	25.5 ± 1.3
		Reversal	6.9 ± 0.2	4337.5 ± 1341.5	9.7 ± 1.3	45.8 ± 5.2	65.8 ± 5.9	13.9 ± 1.5	21.1 ± 0.4

Data in parenthesis indicate % reduction, values in comparison to control. ±SEM; significantly different at * $p < 0.05$; ** $p = 0.01$; *** $p = 0.001$

NS Non significant, *RBC* red blood corpuscles, *WBC* white blood corpuscles, *Hb* haemoglobin, *PCV* packed cell volume, *MCV* mean corpuscular volume, *MCH* mean corpuscular haemoglobin, *MCHC* mean corpuscular haemoglobin concentration

Table 2 Morphological abnormalities (%) in RBC of control and treated mice after sub-chronic and reversal study of distillery soil leachate

Days	Conc. (%)	Treatment	RBC types						
			Normal	Acanthocytes	Spherocyte	Schizocyte	Burr cells/Echinocytes	Tear drop	Target cells
15	5	Subchronic	47.7 ± 8.3**	4.0 ± 1.2^{NS} (+5%)	22.4 ± 5.6* (+28%)	12.6 ± 3.7* (+16%)	2.1 ± 0.9^{NS} (+3%)	2.1 ± 0.4^{NS} (+3%)	9.3 ± 2.1^{NS} (+12%)
		Reversal	97.5 ± 0.3*	0.6 ± 0.1*** (+0.5%)	0.5 ± 0.1** (+0.5%)	0.5 ± 0.1* (+0.4%)	0.3 ± 0.1^{NS} (+0.2%)	0.4 ± 0.1^{NS} (+0.3%)	0.3 ± 0.1^{NS} (+0.2%)
	10	Subchronic	40.0 ± 3.1***	5.8 ± 1.8^{NS} (+10%)	30.0 ± 3.8** (+49%)	12.4 ± 1.6* (+20%)	5.2 ± 1.2^{NS} (+8%)	4.8 ± 1.1* (+8%)	1.8 ± 0.4^{NS} (+3%)
		Reversal	94.7 ± 0.8***	1.5 ± 0.2** (+1%)	1.7 ± 0.4^{NS} (+2%)	1.0 ± 0.3^{NS} (+1%)	0.7 ± 0.2^{NS} (+1%)	0.3 ± 0.1^{NS} (+0.3%)	0.3 ± 0.1^{NS} (+0.3%)
	20	Subchronic	20.5 ± 2.7***	8.8 ± 0.9** (+16%)	30.6 ± 3.8*** **(+54%)**	16.7 ± 1.8*** **(+30%)**	4.2 ± 1.0^{NS} (+7%)	5.8 ± 0.7** (+10%)	11.0 ± 1.1^{NS} (+19%)
		Reversal	88.7 ± 0.77***	3.6 ± 0.4^{NS} (+4%)	3.2 ± 0.4* (+3%)	2.3 ± 0.3* (+2%)	1.2 ± 0.3^{NS} (+1%)	0.6 ± 0.1^{NS} (+1%)	0.6 ± 0.2** (+1%)
30	5	Subchronic	8.8 ± 1.7***	19.8 ± 2.0*** **(+38%)**	20.2 ± 3.6** **(+38%)**	8.1 ± 1.8* (+15%)	35.3 ± 4.4* **(+67%)**	0	5.9 ± 1.1^{NS} (+11%)
		Reversal	97.3 ± 0.4***	0.7 ± 0.2*** (+1%)	0.6 ± 0.1** (+1%)	0.4 ± 0.1** (+0.4%)	0.3 ± 0.1^{NS} (+0.4%)	0.4 ± 0.1^{NS} (+0.4%)	0.4 ± 0.1^{NS} (+0.4%)
Control		Subchronic	96.3 ± 0.5	1.5 ± 0.4 (+2%)	1.1 ± 0.3 (+1.1%)	0.4 ± 0.2 (+0.4%)	0.0	0.8 ± 0.3 (+1%)	0.0
		Reversal	93.6 ± 0.8	2.5 ± 0.3 (+2%)	1.5 ± 0.3 (+1%)	1.0 ± 0.2 (+1%)	1.0 ± 0.2 (+1%)	0.2 ± 0.1 (+0.1%)	0.4 ± 0.1(+0.3%)

Data in parenthesis indicate % reduction, values in comparison to control ±SEM; significantly different at * $p < 0.05$; ** $p = 0.01$; *** $p = 0.001$

NS Non significant

MCH is compatible with macrocytic anemia which may be attributed to several causes, for instance hepatic or pulmonary diseases. Increased MCH values in Algerian mice inhabiting an area contaminated with heavy metals was reported by Nunes et al. (2001). MCHC is an expression of the average concentration of haemoglobin in red blood cells and give the ratio of the weight of haemoglobin to the volume of red blood cells. A decreased MCHC signifies that a unit-volume of packed red blood corpuscles contains less haemoglobin than normal or that haemoglobin has been replaced by erythrocytic stomal materials as in iron deficiency (Fischbach 1984). Our study gets support with the study of Varma and Pratap (2006) who got similar results on mice after administration of distillery effluent.

Distillery soil leachate exposure also adversely affected shapes of red blood corpuscles. Percentage of morphologically abnormal red blood corpuscles (Poikilocytosis) was maximum (11%–67%) during sub-chronic exposure (30 days) at 5% concentration, which is followed by acute treated animals at 20% concentration, while they were very few in control group. Amongst six abnormal types (acanthocytes, spherocytes, schizocytes, burr cells/echinocytes, tear drop and target cells) of red blood corpuscles, burr cells (67%), spherocytes (38%) and acanthocytes (38%) were predominantly found in sub-chronic treated animals, whereas in acute treated animals spherocytes (54%) and schizocytes (30%) were the predominant forms at 20% concentration (Table 2). In the reversal group poikilocytosis decreased and was almost equal to control values (Table 2).

These changes in shape of RBCs may be related to disturbance in osmoregulatory system caused by alteration in cell membrane composition (Shaw et al. 1991). Morphological alterations along with anemic condition might have adversely affected the oxygen carrying capacity of blood, and thereby overall metabolism of the exposed mice.

The analysis of reversal experiment results indicated recovery in values of haematological parameters and reduction in morphological abnormalities in RBC suggesting that experimental animals were returning to their normal life.

Acknowledgments Authors wish to express their sincere thanks to the Head, Department of Zoology, University of Rajasthan, Jaipur, to provide technical support, DBT, New Delhi, for financial support and CSIR, New Delhi for awarding Research Associateship to Dr. Shweta Sharma.

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