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Effects of chemical industry effluents on haematological parameters in mice

Seema Agarwal and DK Agrawal

Abstract
The chemical industry comprises the companies that produce industrial chemicals. Effluents from chemical industries are released abundantly into the environment many times without proper treatment. Limited investigations have been carried out on the impact of chemical industry effluents on the quality of fresh water as well as on health of animals and men. Therefore, present study was done to study the effect of chemical industry effluent on haematological parameters in mice. To study the effect of chemical industry effluent on haematological parameters, two-weeks-old 256 mice procured from Indian Veterinary Research Institute, Izatnagar, Bareilly were randomly divided into four equal groups of 64 mice each viz. control (group-I), R2B vaccine + effluent treated (group-2), effluent treated (group-3) and R2B vaccinated (group-4). The vaccine used was R2B strains given to group-2 and 4 @ 0.1 ml by intraperitoneal route. All the haematological parameters viz. Haemoglobin, Total Erythrocyte Count (TEC), Packed Cell Volume (PCV), Total Leukocyte Count (TLC), lymphocytes, neutrophils, monocytes, eosinophils and basophils count were performed on the day of blood collection at every 15 days interval upto 120 days of experimentation as per the standard method. In the present study, the haematological investigations revealed non significant decrease in haemoglobin, TEC and PCV values up to 75 days of observation in groups 2 and 3 mice in comparison to groups 1 and 4 mice. From 90 to 120 days of observation, a significant decrease in the values of haemoglobin, PCV and TEC was observed in groups 2 and 3 mice in comparison to groups 1 and 4 mice. The decrease in haemoglobin level, TEC and PCV may be because of effect of lead and heavy metals on the haemopoietic system. Heavy metals like lead and cadmium accumulate in erythrocytes and affect haeme synthesis by inhibiting the absorption of iron from the gut.

Keywords: Chemical industry effluents, haematological parameters, mice

Introduction
The chemical industry comprises the companies that produce industrial chemicals. It is central to modern world economy, converting raw materials (oil, natural gas, air, water, metals, minerals) into more than 70,000 different products. Polymers and plastics especially polyethylene, polypropylene, polyvinylchloride, polyethylenetrephthalate, polystyrene and polycarbonate comprise about 80% of the industry's output worldwide. Effluents from chemical industries are released abundantly into the environment many times without proper treatment. Such effluents find their way into the surrounding water bodies and thereby making water unfit for human and animal consumption and life of aquatic organisms. The chemicals present in the effluent are harmful to fauna and flora of receiving water. The residues of the chemicals persist in various food stuffs like cereals, grains, fodder for animals, egg, meat and milk. The heavy metal residues are not only harmful to animals but also causing serious disorders in man by injuring target organs like liver, kidney or immune systems. The alterations in the immune system may result in lower immunocompetance of an individual leading to vaccinal failures, occurrence of outbreak of various diseases. These effluents not only causing the alteration in the immune system but also causing the alteration in haematological parameters. Keeping in view the nature of chemical industry effluent, a survey was conducted around chemical industries. The impact of such effluent on animal health is to be studied using a mice model.

Materials and Methods
For the proposed study, samples of effluent were collected from and nearby effluent passing areas through the naala near Jubilant organosys (Gajraula). The samples of effluent were collected and brought to the laboratory. The toxicity of the effluent was studied in laboratory animals (mice) by giving effluent water *ad libidum* for four months of duration.

To study the effects of chemical industry effluent in haematological parameters on mice, 256 mice of 2 weeks age were procured from laboratory animal experimental house, IVRI, Izatnagar, Bareilly. Before keeping the mice, the experimental house was thoroughly cleaned with water and then with 1% phenyl solution. Cages, wateres and feeders after washing with water and phenyl solution were cleaned with potassium permanganate solution. Total 256 mice were randomly divided into four groups of 64 mice each viz. control (group-I), R2B vaccinated (group-2), effluent treated (group-3) and R2B vaccine + effluent treated (group-4). Animals were anesthetized and blood was collected via cardiac puncture with heparinzed syringe. All the hematological parameters viz. hemoglobin, packed cell volume, total leukocyte count, total erythrocyte count and differential leucocyte count were performed on the day of blood collection as per the standard method described by Benjamin (2005) [3].

Results

The values of different haematological parameters i.e. hemoglobin, PCV, TEC, TLC, lymphocytes, neutrophils, monocytes, eosinophils and basophils count showed non-significant change throughout the duration of experiment in groups 1 and 4 mice in comparison to groups 2 and 3 mice. The values of different haematological parameters are shown in tables 1, 2, 3, 4, 5 and 6.

In group 2 and in group 3 the haemoglobin level at 15 day was 14.65±0.31 g/dl and 14.24±0.23 g/dl which varied non-significantly up to 75th day. However, significant decrease in haemoglobin level was observed on 90th day ($p\leq0.05$), 105th day ($p\leq0.05$) and 120th day ($p\leq0.05$) as compared to groups 1 and 4 mice and the values were 12.8±0.31, 12.48 ±0.13, and 11.48±0.20 g/dl respectively in group 2 and values in group 3 mice were 12.2±0.18, 11.22 ±0.30 and 11.1±0.20 g/dl, respectively.

In group 2 and in group 3 mice, the PCV level at 15 day was 45.0±1.2% and 43.0±1.25% which varied non-significantly up to 75th day. However, significant decrease was observed on 90th day ($p\leq0.05$), 105th day ($p\leq0.05$) and 120th day ($p\leq0.01$) as compared to groups 1 and 4 and the values were 42.62±3.25, 40.92 ±2.00 and 40.07±2.08%, respectively in group 2 and in group 3 on these days were 41.1±2.25, 41.0±2.41, and 40.92±3.89%, respectively.

In group 2, the total erythrocyte count at day 15 was 8.84±0.09 × 10^6 /µl and in group 3, the total erythrocyte count after 15 day was 8.85±0.15 × 10^6 per µl, which varied non-significantly up to 75th day. However, significant decrease was observed on 90th day ($p\leq0.05$), 105th day ($p\leq0.05$) and 120th day ($p\leq0.05$) as compared to groups 1 and 4 and the values in group 2 on these days were 7.78±0.09, 7.73±0.12 and 7.69±0.13 ×10^6/µl, respectively and the values in group 3 on these days were 7.59±0.06, 6.55±0.06, and 6.42±0.14 × 10^6 /µl, respectively.

In group 2 and in group 3 the total leukocyte count at day 15 was 8.745±0.191 × 10^3 per µl and 8.743±0.001 ×10^3 per µl which varied non-significantly up to 75th day. However, significant decrease was observed on 90th day ($p\leq0.05$), 105th day ($p\leq0.05$) and 120th day ($p\leq0.05$) as compared to the values in groups 1 and 4 and the values in group 2 on these days were 6.64±0.040, 6.63±0.007, and 6.62±0.008 × 10^3 /µl, respectively. The total leukocyte count values in group 3 on days 90, 105 and 120 were 6.38±0.398, 6.63±0.143, and 6.63±0.406 × 10^3 /µl, respectively.

In group 2 and in group 3, the lymphocyte count varied non-significantly up to 75th day. However, significant decrease in its value was observed on 90th day ($p\leq0.05$), 105th day ($p\leq0.05$) and 120th day ($p\leq0.01$) when compared with the values in groups 1 and 4 and the lymphocyte counts were 67.75±0.62, 67.5±0.64, and 67.5±0.640%, respectively in group 2.

In group 2, the neutrophil count at day 15 was 25.50±0.64% that varied non-significantly up to 75th day. However, significant decrease was observed on 90th ($p\leq0.01$), 105th day ($p\leq0.05$) and 120th day ($p\leq0.05$) as compared to the values in groups 1 and 4 and the values in group 2 were 24.25±0.64, 24.0±0.40 and 23.5±0.64% respectively, on these days. The neutrophil values were 24.10±0.47, 24.0±0.40 and 23.5±0.64%, respectively, in group 3 on day 90, 105 and 120.

The monocyte, eosinophil and basophil count showed non-significant change throughout the experiment in all the groups.

Table 1: Haemoglobin concentration (Hb gm%) in experimental mice in different groups at 15 days interval upto 120 days of observation.

Days of Parameters	Control (Gp-1)	Effluent+ vaccine treated (Gp-2)	Effluent treated (Gp-3)	Vaccine treated (Gp-4)
15	14.4±0.61	14.65±0.31	14.24±0.23	14.5±0.13
30	14.58±0.46	14.34±0.61	13.3±0.31	14.9±0.41
45	14.5±0.61	13.93±0.41	13.2±0.21	14.8±0.31
60	14.67±0.36	13.41±0.38	12.94±0.61	14.2±0.1
75	15.1±0.63	13.1±0.13	12.4±0.12	15.1±0.31
90	15.2±0.68	12.8±031**	12.2±0.18*	15.3±0.36
105	15.67±0.63	12.48±0.13*	11.22±0.30*	15.0±0.10
120	15.9±0.10	11.48±0.20*	11.1±0.20*	15.1±0.10

* $p<0.05$
** $p<0.01$

Table 2: Packed Cell Volume (PCV %) in experimental mice in different groups at 15 days interval upto 120 days of observation

Days of Parameters	Control (Gp-1)	Effluent+ vaccine treated (Gp-2)	Effluent treated (Gp-3)	Vaccine treated (Gp-4)
15	45.5±0.22	45.0±1.2	43.0±1.25	44.9±2.25
30	46.72±0.91	44.50±1.5	42.65±2.5	43.72±2.66
45	45.25±3.25	43.5±1.75	42.275±2.82	46.6±1.82
60	46.5±1.29	43.25±0.285	42.20±1.75	43.0±3.25
75	44.5±0.52	42.65±2.59	42.1±0.59	43.25±0.29
90	46.65±1.52	42.62±3.25*	41.1±2.25*	44.7±2.14
105	47.97±2.01	40.92±2.0*	41.0±2.41*	44±3.1
120	46.07±2.08	40.07±2.08**	40.92±3.89*	45±2.41

*p<0.05
**p<0.01

Table 3: Total Erythrocytes Count (TEC x 10^6/µl) in experimental mice in different groups at 15 days interval upto 120 days of observation

Days of Parameters	Control (Gp-1)	Effluent+ vaccine treated (Gp-2)	Effluent treated (Gp-3)	Vaccine treated (Gp-4)
15	8.87±0.10	8.84±0.09	8.85±0.15	8.79±0.08
30	8.87±0.08	8.46±0.15	8.79±0.09	8.84±0.09
45	8.85±0.08	8.8±0.58	8.70±0.12	8.87±0.047
60	8.8±0.10	8.82±0.09	8.69±0.13	8.57±0.236
75	8.7±0.14	8.8±0.12	8.62±0.06	8.69±0.162
90	8.7±0.12	7.78±0.09*	7.59±0.06*	8.61±0.133
105	8.60±0.26	7.73±0.12*	6.55±0.06*	8.70±0.184
120	8.79±0.113	7.69±0.13*	6.42±0.14*	8.80±0.06

*p<0.05

Table 4: Total Leukocyte Count (TLC x 10^3/µl) in experimental mice in different groups at 15 days interval upto 120 days of observation

Days of Parameters	Control (Gp-1)	Effluent+ vaccine treated (Gp-2)	Effluent treated (Gp-3)	Vaccine treated (Gp-4)
15	8.75±0.104	8.745±0.191	8.743±0.001	8.850±0.132
30	8.165±0.404	8.665±0.147	8.742±0.01	8.875±0.149
45	8.512±0.185	8.664±1.63	8.642±0.0	8.887±0.042
60	8.60±0.227	8.660±0.040	8.641±0.010	8.850±0.132
75	8.875±0.149	7.660±0.040	7.638±0.135	8.875±0.149
90	8.775±0.265	6.640±0.040*	6.387±0.398*	8.980±0.146
105	8.875±0.042	6.635±0.0.007*	6.635±0.143*	8.870±0.004
120	8.665±0.147	6.620±0.008*	6.633±0.406*	8.925±0.018

*p<0.05

Table 5: Lymphocyte (L %) in experimental mice in different groups at 15 days interval upto 120 days of observation

Days of Parameters	Control (Gp-1)	Effluent+ vaccine treated (Gp-2)	Effluent treated (Gp-3)	Vaccine treated (Gp-4)
15	70.52±0.64	69.0±0.40	68.5±0.64	70.0±0.40
30	70.25±0.85	68.75±0.85	48.25±0.47	69.75±0.47
45	68.75±0.64	68.75±0.85	68.30±0.40	70.50±0.64
60	69.57±0.64	68.5±0.40	67.75±0.47	70.25±0.85
75	69.0±0.64	68.0±0.40	67.5±0.64	70.0±0.40
90	69.5±0.64	67.75±0.62*	67.0±0.40*	69.75±0.41
105	69.2±0.47	67.5±0.64*	67.03±0.41*	71.0±0.40
120	69.0±0.40	67.5±0.64**	66.7±0.85**	70.8±0.64

*p<0.05
**p<0.01

Table 6: Neutrophil (N %) in experimental mice in different groups at 15 days interval upto 120 days of observation

Days of Parameters	Control (Gp-1)	Effluent+ vaccine treated (Gp-2)	Effluent treated (Gp-3)	Vaccine treated (Gp-4)
15	25.75±0.49	25.5±0.64	25.25±0.85	25.5±0.64
30	25.5±0.28	25.25±0.47	25.25±0.47	25.25±0.47
45	25.0±0.40	25.25±0.85	25.0±0.70	25.25±0.62
60	24.75±0.47	25.0±0.70	24.75±0.65	25.0±0.40
75	24.5±0.64	24.75±0.47	24.5±0.64	25.0±0.91
90	24.75±0.47	24.25±0.64**	24.10±0.47**	24.75±0.47
105	25.0±0.40.	24.0±0.40*	24.0±0.40*	24.25±0.47
120	24.75±0.47	23.5±0.64*	23.5±0.64*	24.0±0.40

*p<0.05

Discussion

Murata *et al.* (1970) [13] recorded decrease Hb, PCV and RBC count in three months old rats when exposed to chlorinated water. Blakley (1982) [4] during chronic copper toxicity in cows recorded poikilocytosis, increased red cell fragility, decreased packed cell volume, haemoglobinemia and icterus. Burrows and Borchard (1982) [5] also reported decrease in PCV and TEC values in ponnies when given smelter effluent contaminated hay with lead acetate. Varadarajan *et al.* (1991) [17] also reported decreased haemoglobin, TEC and PCV values in cows fed contaminated pasture near Avantipuram sewage irrigated area in Tamil Nadu. The above findings are in accordance with the observations of Cho *et al*. (1994) [7] who observed subnormal haemoglobin and PCV level in experimental rats fed cadmium for two months and also with the findings of Mishra (2002) [11] and Amarnath *et al*. (2004) [1] who noticed decreased haemoglobin, TEC and PCV values in cadmium exposed rabbits and heavy metal toxicity in animals, respectively. Sujatha and Sriraman (1996) [19] recorded significant decrease in the mean TEC, Hb, PCV and TLC at higher blood lead concentration and increase in erythrocyte fragility on lead toxicity in buffaloes. Upadhyay (2007) [18] also recorded decrease values of haemoglobin, TEC and PCV in pulp and paper mill effluent exposed mice. In the present study, the decrease in haemoglobin level, TEC and PCV may be because of effect of lead and heavy metals on the haemopoietic system. Heavy metals like lead and cadmium accumulate in erythrocytes and affect haeme synthesis by inhibiting the absorption of iron from the gut (Monica *et al*., 2003 and Nolan and Shaikh, 1992) [12, 14]. These findings also indicate poor feed intake, improper utilization of the nutrient and metabolic disturbances as recorded by Benjamin (2005) [3]; Jones *et al*. (1997) [8] and Radostits *et al*. (2000) [15].

The present study also showed significant decrease in TLC and lymphocyte percentage values from 75 to 120 days of observation in groups 2 and 3 mice. The similar findings were also recorded by Varadarajan *et al.* (1991) [17]; Avram *et al.* (1995) [2]; Sujatha and Sriraman (1996) [19]; and Amarnath *et al*. (2004) [1] in heavy metal toxicity in animals. Teijon *et al*. (2003) [16] also observed decreased values of TLC and lymphopenia in rats given contaminated lead acetate water. Chamraju (2007) [6] and Manjunath (2007) [10] also observed the decrease in TLC from 60th day onwards in Brass and distillery effluent treated mice. Similar results were also recorded by Upadhyay (2007) [18] during the study of pulp and paper industry effluent effect in mice. The decrease in TLC is generally encountered in chronic exposure to chemical agents like lead, cadmium, etc., which act as metabolic poisons in animals (Benjamin, 2005) [3].

Lymphopenia in the present study, may be correlated with the associated leukopenia as a result of decreased proliferation of lymphocytes and immuno-suppression. Amarnath *et al*. (2004) also recorded leukopenic lymphopenia in rabbits exposed to oral cadmium chloride treatment.

The neutrophil percentage in the present study revealed significant decrease in group 2 and 3 on day 105 and 120 during the investigation period. Similar findings were also reported by Lynch *et al*. (1976) [9] and Teijon *et al*. (2003) [16] in lead and cadmium toxicity in mice. The above findings are in agreement with the finding of Upadhyay (2007) [18] in mice fed paper and pulp industry effluent.

Conclusion

Chemical industry effluent causes significant chronic health impact on animals and therefore, constant monitoring is required for the effluent to be released only after proper treatment to prevent ill effects on the animal as well as human health.

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