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Effects of Chemical Industry Effluents on Biochemical Parameteres in Mice

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ABSTRACT: The present study was conducted to study the effect of chemical industry effluent on biochemical parameters in mice. To study the effect of chemical industry effluent on biochemical 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 biochemical parameters viz. total protein, albumin, globulin, albulin globulin ratio, bilirubin, ALT, AST, creatinine and Blood Urea Nitrogen were performed on the day of blood collection at every 15 days interval upto 120 days of experimentation as per the standard method. It was observed total protein, albumin, globulin were reduced significantly in chemical industry effluent treated group. The Blood Urea Nitrogen, creatinine, AST and ALT showed significantly higher level in chemical industry effluent treated group in comparison to controls. A significant decrease in total protein, albumin, globulin, and increase in Blood Urea Nitrogen, creatinine, AST and ALT levels could be attributed to hepatic and kidney damage, generally caused by systemic toxicity of heavy metals present in the industrial effluent.

Key words: chemical industry effluents, biochemical parameters, mice.

I.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 biochemical 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.

II.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 on biochemical parameters in mice, 256 mice of 2 weeks age were procured from Laboratory Animal Research (LAAR)

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Section, 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 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. The mice were housed under ideal conditions of hygiene and management. The room temperature was maintained about 30^0C in summer with the help of air cooler. The mice of effluent treated and vaccine + effluent treated groups were given ad libidum effluent water for drinking. Mice of all groups were maintained on the feed supplied by the Experimental Animal House of College of Veterinary & Animal Sciences, Pantnagar. Mice from each group were sacrificed at 15, 30, 45, 60, 75, 90, 105 and 120 days of experimentation for estimation of biochemical parameters. Animals were anesthetized and blood was collected via cardiac puncture with heparinized syringe. After collecting blood, serum was obtained and stored at -20^0C. All the biochemical parameters under the study (total protein, albumin, blood urea nitrogen, total bilirubin and creatinine) were analyzed with Auto analyzer (ACE/Next)$^{-TM}$ clinical chemistry system and their kits manufactured by Alfa Wassermann BV, Pompmolenlan. 24, 3447 GK, Woerden, Netherlands). Serum enzymes (alanine amino transferase and aspartate ammo transferase) were determined spectrophotometrically on the day of serum collection using kits manufactured by AXIS Diagnostic and Biotech. Ltd, plot No 66, sector -18, Huda, Gurgaon, India. The data obtained from different studies was analyzed as per the methods described by Snedecor and Cochran**.**

III. RESULTS

The different biochemical parameters, i.e. total protein, albumin, globulin, albumin globulin ratio, bilirubin, ALT, AST, creatinine and blood urea nitrogen showed non-significant change throughout the experiment in groups 1 and group 4. The values of different biochemical parameters are shown in tables 1, 2, 3, 4, 5, 6, 7, 8 and 9 along with figures 1, 2, 3, 4, 5, 6, 7, 8 and 9. In group 2, the total protein level at day 15 was 6.61±0.178/dl and decreased non-significantly up to 75th day. However, significant decrease was observed on 90th day ($P\leq0.05$), 105th day ($P\leq0.01$) and 120th day ($P\leq0.05$) as compared to the values in groups 1 and 4 and the values in group 2 were 5.40±0.12, 5.39±0.122 and 5.10±0.42 g/dl, respectively.

In group 3, the total protein level after 15 day was 6.67±0.42 g/dl and decreased 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 ($P\leq0.05$) when compared with values in groups 1 and 4. The total protein values were 5.30±0.299, 5.27±0.776, and 5.10±0.675 g/dl, respectively, in group 3 on day 90,105 and 120.

In group 2 mice, the albumin level after 15 day was 3.2±0.086 g/dl, which varied non-significantly up to 75th day. However, significant decrease was observed on 90th ($P\leq0.05$), 105th day ($P\leq0.05$) and 120th day ($P\leq0.01$) as compared to groups 1 and 4 and the values were 2.90±0.22, 2.88±0.408, and 2.87±0.361 g/dl, respectively.

In group 3, the albumin level at day 15 was 3.15±0.024 g/dl which varied non-significantly up to 75th day. However, significant decrease was observed on 90th day ($P\leq0.01$), 105th day ($P\leq0.01$) and 120th day ($P\leq0.05$) as compared to groups 1 and 4 and the in group 3 on these days were 2.79±0.149, 2.44±0.167, and 2.26±0.074 g/dl, respectively.

In group 2, the globulin level at day 15 was 2.91±0.24 g/dl and it varied non-significantly up to 75th day. However, significant decrease was observed on 90th day ($P\leq0.01$), 105th day ($P\leq0.01$) and 120th day ($P\leq0.01$) as compared to groups 1 and 4 values and the values in group 2 were 2.51±0.11, 2.50±0.43 and 2.46±0.48 g/dl, respectively, on these days.

In group 3, the globulin level at 15 day was 3.02±0.58 g/dl and its value varied non-significantly up to 75th day. However, significant decrease was observed on day 90 ($P\leq0.05$), day105 ($P\leq0.05$) and day120 ($P\leq0.05$) as compared to the values in groups 1 and 4 on these days. Globulin levels in group 3 were 2.44±0.30, 2.43±0.85 and 2.41±0.20 g/dl respectively, on day 90, 105 and 120.

In all the groups, the concentrations of albumin-globulin ratio, and total bilirubin varied non-significantly throughout the duration of the experiment (Table 4 and 5).

In group 2, the ALT level after 15 day was 22.5±8.8 U/l and it varied non-significantly up to 75th day. However, significant increase was observed on 90th day ($P\leq0.05$), 105th day ($P\leq0.05$)

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and 120th day (P≤0.01) as compared to groups 1 and 4 and the values were 50.5±[1]4, 70.1±[1]4 and 7[1]5.0±[1]3 U/l, respectively in group 2 on these days.

At day 15, the ALT level in group 3 was 24.1±3.1 U/l. Its level did not vary significantly up to 75th day. However, significant increase in ALT level was observed on day 90 (P≤0.05), day 105 (P≤0.05) and day 120 (P≤0.05) in group 3 as compared to groups 1 and 4 values. In group 3, ALT level on day 90, 105 and 120 was 55.3±[1]2, 60.4±4.9 and 65.0±[1]9 U/l, respectively. The AST level in group 3 at day 15 was 74.4±4.10 U/l and it revealed non-significant change up to 75th day. However, significant increase was observed in AST level on 90th (P≤0.05), 105th day (P≤0.01) and 120th day (P≤0.01) in group 3 as compared to the values in groups 1 and 4 on these days. The value of AST on day 90, 105 and 120 were 104.0±3.3, 124.0±4.1 and 148.0±8.01 U/l, respectively in group 3.

At day 15, the creatinine level in group 2 was 0.762±0.017 mg/dl and its level differed non-significantly up to 75th day. However, significant increase was observed on 90th day (P≤0.05), 105th day (P≤0.01) and 120th day (P≤0.05) in group 2 as compared to respective values in groups 1 and 4. The concentration of creatinine on days 90, 105 and 120 in group 2 were 0.967±0.031, 0.978±0.03 and 0.979±0.035 mg/dl, respectively.

In group 3, the creatinine concentration on day 15 was 0.759±0.030 mg/dl which varied non-significantly up to 75th day. There was significant increase in its level on day 90 (P≤0.05), day 105 (P≤0.05) and day 120 (P≤0.05) as compared to groups 1 and 4 values on respective days. Its values were 0.955±0.066, 0.954±0.039 and [1]0±0.042 mg/dl, respectively on these days in group 3 mice.

In group 2, the blood urea nitrogen level (table 9) at 15 day was 44.3±2.5 mg/dl and it varied non-significantly up to 75th day. However, significant increase was observed on 90th day (P≤0.05), 105th day (P≤0.05) and 120th day (P≤0.01) in group 2 when compared to the values in groups 1 and 4 and the values of BUN on days 90, 105 and 120 were 76.0±[1]09, 78.0±8.1 and 80.1±2.15 mg/dl, respectively in group 2.

The value of blood urea nitrogen at day 15 in group 3 was 44.1±5.4 mg/dl and it varied non-significantly up to 75th day. However, significant increase was observed on, day 90 (P≤0.05), 105 (P≤0.05) and 120 (P≤0.01) as compared to values in groups 1 and 4 on these days. The values were 69.0±[1]01, 79.0±5.1and 82.5±[1]5 mg/dl, respectively, in group 3 on the respective days.

In group 2 mice, the AST level at 15th day was 75.5±5.50 U/l and it varied non-significantly up to 75th day. However, significant increase was observed on 90th (P≤0.05), 105th day (P≤0.01) and 120th day (P≤0.01) in group 2 as compared to AST values in groups 1 and 4. The values of AST in group 2 were 108±4.3, 127±3.7 and 150±4.1 U/l, respectively.

In the present study, a significant decrease in the value of total protein, albumin and globulin was observed from day 90 to day 120 of observation in groups 2 and 3 mice in comparison to groups 1 and 2 mice. **Varandarajan et al. (1991)** recorded lower values of serum globulin in cows fed contaminated pasture near Avantipuram area in Tamil Nadu.

A significant increase in BUN, creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values was observed from 90th day onwards in groups 2 and 3 mice up to 120 day of observation. The decrease in total protein, albumin and globulin along with increase in BUN, creatinine, ALT and AST have also been reported by **Amarnath et al. (2004)** in cadmium exposed mice. **Joshi et al. (2007)** and **Patel et al. (2007)** reported increase in creatinine, gamma glutamyl transferase and alkaline phosphatase in lead acetate fed rats and cadmium chloride exposed rats respectively. Similar results were also reported by **Upadhyay (2007)** in mice exposed to pulp and paper industry effluent.

A significant decrease in serum total protein, albumin and globulin and increase in BUN, creatinine, ALT and AST levels could be attributed to hepatic and kidney damage, generally caused by systemic toxicity of heavy metals present in the industrial effluent **(Benjamin, 2005).**

BUN and creatinine are the metabolic products of the body which are primarily excreted through the kidney. Any factor affecting the kidney is likely to enhance the level of these components in the blood. In the present study the increased level of BUN and creatinine in the present study is an indicative of deranged kidney due to accumulation of high concentration of heavy metals in the kidney **(Jubb 1985; Jones et al., 1997** and **Radostits et al., 2000).** According to **Goyer** and **Clarkson (1996)** liver and kidney are the main targetted organs by most of the heavy metals.

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ALT and AST enzymes primarily are specific for liver functioning **(Kaneko, 1997).** Therefore, high activities of these enzymes in the present study might be correlated with the involvement of the liver and kidney as a result of nephrotoxic and hepatotoxic effect of heavy metals **(Liu et al., 1992).** Heavy metals are among the main pollutants causing water pollution, affecting aquatic ecosystem and causing harmful effects on animals and human beings **(Goldblatt** and **Anthony, 1983)**. Toxic effect of these industrial heavy metals is damage to different organs at cellular and molecular level **(Choudhary, 1958).**

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Table [1] Total Protein (TP gm/dl) 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	6.61±0.54	6.61±0.178	6.67±0.42	6.75±0.21
30	6.90±0.54	5.60±0.119	5.39±0.37	6.81±0.41
45	6.12±0.246	5.57±0.663	5.38±0.47	6.92±0.15
60	6.24±0.141	5.49±0.344	5.35±0.264	6.98±0.56
75	6.027±0.679	5.41±0.124	5.32±0.409	6.98±0.62
90	6.04±0.233	5.4±0.12*	5.30±0.299*	7.06±0.22
105	6.22±0.09	5.39±0.122**	5.27±0.776*	7.08±0.01
120	6.39±0.186	5.10±0.42*	5.10±0.675*	7.23±0.12

* P<0.05
** P<0.01

Table 2 : Albumin (gm/dl) 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	3.29±0.20	3.20±0.086	3.15±0.024	3.16±0.239
30	3.14±0.43	3.17±0.267	3.34±0.198	3.55±0.212
45	3.18±0.09	3.08±0.69	2.8±0.408	3.76±0.151
60	3.30±0.54	3.05±0.21	3.04±0.234	3.522±0.384
75	3.32±0.39	3.01±0.21	2.89±0.077	3.58±0.18
90	3.34±0.21	2.90±0.22*	2.79±0.149**	3.84±0.259
105	3.365±0.19	2.88±0.408*	2.44±0.167**	3.47±0.08
120	3.55±0.18	2.87±0.361**	2.26±0.074*	3.54±0.475

* P<0.05
** P<0.01

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Table 3 : Globulin (gm/dl) 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	2.32±0.287	2.91±0.24	3.02±0.58	2.70±0.21
30	2.75±0.394	2.93±0.11	2.83±0.20	3.51±0.53
45	2.94±0.299	2.95±0.13	2.58±0.50	3.16±0.30
60	2.94±0.546	2.84±0.54	2.91±0.26	3.45±0.34
75	2.70±0.471	2.60±0.16	2.50±0.38	3.08±0.42
90	2.70±0.437	2.51±0.11**	2.44±0.30*	2.96±0.22
105	2.86±0.147	2.50±0.43**	2.43±0.85*	3.53±0.64
120	2.75±0.322	2.46±0.48**	2.41±0.20*	3.25±0.56

* P<0.05
** P<0.01

Table 4 : A:G ratio 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	[1]02±0.1	[1]04±0.04	[1]03±0.0	[1]01±0.02
30	[1]04±0.0	[1]05±0.0	[1]02±0.0	[1]0±0.02
45	[1]01±0.02	0.99±0.04	0.99±0.03	[1]0±0.01
60	0.10±0.02	0.94±0.0	0.99±0.08	0.95±0.01
75	0.99±0.05	0.95±0.0	0.94±0.0	0.98±0.01
90	0.93±0.01	[1]01±0.02	[1]02±0.41	0.91±0.05
105	0.99±0.0	0.98±0.02	0.99±0.05	0.90±0.02
120	0.94±0.02	[1]0±0.02	[1]02±0.03	0.99±0.09

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Table 5: Total Bilirubin 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	0.41±0.02	0.312±0.025	0.357±0.21	0.362±0.011
30	0.44±0.006	0.425±0.029	0.395±0.275	0.452±0.021
45	0.432±0.02	0.417±0.011	0.432±0.10	0.455±0.015
60	0.487±0.02	0.460±0.022	0.440±0.009	0.467±0.016
75	0.485±0.018	0.467±0.016	0.457±0.03	0.417±0.029
90	0.505±0.006	0.332±0.028	0.550±0.047	0.357±0.01
105	0.539±0.045	0.510±0.043	0.420±0.02	0.462±0.023
120	0.452±0.064	0.58±0.013	0.597±0.016	0.490±0.01

Table 6 : BUN (mg/dl) 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	44.5±2.1	44.3±2.5	44.1±5.4	44.5±0.39
30	44.6±[1]2	45.0±[1]1	45.1±[1]0	45.0±2.2
45	48.1±[1]09	50.1±0.85	50.3±8.3	48.1±0.84
60	48.2±[1]05	55.5±0.99	59.1±0.12	48.0±3.21
75	46.0±[1]19	64.0±[1]65	68.0±3.1	48.3±2.64
90	46.5±[1]07	76.0±[1]09*	69.0±[1]01*	45.1±2.2
105	48.0±[1]65	78.0±8.1*	79.0±5.1*	50.1±3.1
120	49.0±[1]45	80.1±2.15**	82.5±[1]5**	50.1±3.4

* P<0.05
** P<0.01

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Table 7 : Creatinine (mg/dl) 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	0.75±0.02	0.762±0.017	0.795±0.030	0.712±0.53
30	0.745±0.034	0.780±0.013	0.800±0.010	0.732±0.071
45	0.804±0.020	0.795±0.042	0.820±0.50	0.860±0.046
60	0.777±0.05	0.810±0.083	0.840±0.052	0.785±0.054
75	0.640±0.01	0.835±0.041	0.860±0.061	0.080±0.057
90	0.795±0.03	0.967±0.031*	0.955±0.066*	0.073±0.06
105	0.762±0.03	0.978±0.03**	0.954±0.039*	0.752±0.039
120	0.787±0.06	0.979±0.035*	[1]00±0.042*	0.710±0.035

* P<0.05
** P<0.01

Table 8 : AST (U/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	75.9±4.5	75.5±5.5	74.4±4.1	93.5±3.1
30	77.2±2.61	80.1±4.03	74.5±4.3	78.0±4.1
45	75.9±0.13	84.3±4.3	8[1]5±6.6	75.4±5.3
60	74.4±6.1	109.2±5.1	98.8±4.1	75.0±5.1
75	80.0±5.3	95.0±4.1	99.0±3.1	8[1]6±4.6
90	80.5±4.1	108.0±4.3*	104.0±3.3*	86.0±3.1
105	75.0±5.3	129.0±3.7**	124.0±4.1**	85.1±2.1
120	85.5±4.4	150.0±4.1**	148.0±8.01**	85.1±3.1

* P<0.05
** P<0.01

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Table 9 : ALT (U/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	24.4±2.1	22.5±8.8	24.1±3.1	23.0±5.1
30	24.0±[1]4	25.0±2.03	25.0±[1]9	24.5±[1]8
45	25.4±8.3	30.4±0.52	32.0±4.0	25.8±3.2
60	25.9±[1]4	35.4±5.2	35.1±4.2	35.0±[1]8
75	24.7±2.5	45.1±2.9	45.2±[1]9	2[1]5±2.0
90	25.5±2.6	50.5±[1]4*	55.3±[1]2*	25.8±2.6
105	25.6±3.1	70.1±[1]4*	60.4±4.9*	26.5±3.4
120	26.2±4.1	7[1]5±[1]3**	65.0±[1]9*	30.5±[1]8

* P<0.05
** P<0.01