Alterations in Biochemical and Histopathological Profile of Liver in Distillery Soil Leachate Treated Swiss Albino Mice (*Mus Musculus L.*)

Subhasini Sharma*, Kalpana Sharma*, Nivedita Yadav* and K.P. Sharma

***Department of Zoology, University of Rajasthan, Jaipur.**
Department of Botany, University of Rajasthan, Jaipur.

Summary

Sub acute toxicity of different concentrations (5%, 10% and 20%) was studied in Swiss albino mice. Profound changes in the architecture of liver were seen viz. loss of radial arrangement of hepatocytes, dilation of sinusoids, cellular vacuolization and hypertrophy of the central vein, focal histocytic, focal granulomatus inflammation, binucleated hepatic cells, karyolysis, pycnosis and necrosis. Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Bilirubin and Alkaline Phosphatase values increased significantly whereas an opposite trend decrease was noted in the value of Total Protein, albumin and globulin except in 5% concentration group where increase trend was noted for total protein. Post-treated animals did not show any recovery, in comparison to control mice.

Thus, present study revealed toxic effects of distillery soil leachate, providing a basis for documental potential regarding clinical, pathological and biochemical assessment.

Key words: Distillery soil leachate; Toxicity; Albino mice; Serum biochemistry

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Dr. Subhasini Sharma (Associate Professor), C-141A- Malviya nagar,Jaipur-17
Rajasthan, India. E-mail:subhasini2@gmail.com, subhasini_sharma@yahoo.com
Tel: 91-9414054120

Introduction

Nearly, 70% population of India lives in villages, which is directly or indirectly concerned with farming. Economic development of the country is based on the yield from agricultural fields. Sugar industry is one of the main agro-based industries in the country. There are about 579 sugar mills and 285 distilleries in India[1]. Molasses is the chief source for the production of alcohol in distilleries[1].

About 40 billion litres of wastewater annually discharged by distilleries, known as spent wash is dark brown in colour (due to melanoidins), acidic in nature (pH 3.94-4.30) , EC (30-45 ds/m), biological oxygen demand (BOD: 50,000-80,000 mg/L), Chemical Oxygen Demand (COD: 25,000-30,000 mg/L) [2,3,4]. The values of these parameters are several folds greater than the discharge limits as prescribed by CPCB[5]. Though spent wash generally does not contain any toxic metals, but due to high BOD, COD along with other organic compounds and unpleasant odour, are likely to deteriorate soil and environmental health. This deterioration caused after disposal of effluents on land and soil of surrounding farmlands is a relatively new area of research.

Hence, discharge of spent wash is a serious problem. Several toxicological studies of distillery wastewater were made on animals, especially on fish[6]. However, information is scarce on its adverse effects on terrestrial animals. In our laboratory, the toxic effects of distillery soil leachate were made on haemopoetic system of Swiss albino mice [7].

In continuation of it, present work has been undertaken to assess the impact of distillery soil leachate on vital organs (liver) of Swiss albino mice.

Material and Methods

Distillery soil collected from the crop field near Behrod, Alwar district (Rajasthan, India) was dried in the sun. It was ground and passed through stainless sieve of 2 mm mesh size. Soil leachate prepared by stirring it in distilled water (1:1) for about 24h, and allowing soil suspension to stand still for almost 4h in a measuring cylinder. Clear supernatant was pumped out and filtered by passing through the whatman filter paper. Now prepared soil leachate was considered 100%, thereafter different concentrations viz, 5%, 10%,and 20% were made by adding potable water, on the basis of the LD 50 (12.1) calculated by using the COMPAQ personal computer BASIC version 1.13. There physico-chemical characteristics were made according to the APHA [8].

Animal's Model

Swiss albino mice (*Mus musculus L.*) (age, 45-50 days; weight,35-45 g) from an inbred colony were acclimatized for 10 days prior to experiment. Four mice were kept in the polypropylene cages at 25±3ºc, 40%- 60% relative humidity and 12h alternate light: dark cycle as per guidelines of the Institutional Ethical Committee. They are fed on mice feed from Hindustan Lever Ltd, India and the potable water was provided ad libitum. These animals were divided into four groups having ten animals each.

Group 1: Standard Feed + Potable water
Group 2: Standard Feed + 5% distillery soil leachate (15 days)
Group 3: Standard Feed + 10% distillery soil leachate (15 days)
Group 4: Standard Feed + 20% distillery soil leachate (15 days)

Food and water intake

The food and water intake of an animal has been calculated by substracting its left over amount from the initial amount after 24 hours.

Initial amount- left over amount = consumed amount

Body and organ weight

Body weight and weight of different organs: Initial and final weight of both control and treated animals were recorded prior and after the completion of the experiment. After 15 days 5 animals of each experiment were sacrificed. Blood was collected by the cardiac puncture and serum was separated [9].

Serum biochemistry

The serum biochemical parameters were estimated by using auto analyzer (Transia Mumbai, India) viz: Asparate aminotransferase (AST), alanine aminotransferase (ALT) bilirubin, alkaline phosphatase, protein, albumin and globulin[10].

Histopathology of Liver

Liver from the autopsied animals were excised out and fixed in the Bouins fixative. Sections of 5µ thickness were cut and stained [11].

Reversal Study

Reversal (Post-treatment) study was performed on the remaining 5 mice of each group by providing potable water for 45 days following the same procedure.

Statistics

The data were subjected to the statistical analysis expressed as mean± SEM (Standard error of mean). Statistical significance between the control and experimental data were calculated by student 't' test and one way of analyzing of Variance (ANOVA).

Results

Swiss albino mice were treated with different concentrations (5-20%) of distillery soil leachate for15 days showed decline in food and water intake (7-10%), (27-45%) along with decreased body weight (1-10%) and the weight of liver (10-14%) (Table1).

Tab.1. Food and water intake, body and liver weight of distillery soil leachate treated and reversal group.

Parameter	Control	15 Days		
		20 %	10 %	5 %
Food intake	5.61±0.005	5.2±0.05** (7%)	5.1±0.05*** (9%)	5.05±0.03*** (10%)
Water intake	13.9±0.23	7.6±0.35*** (45%)	10.2±0.12*** (27%)	9.12±0.03*** (34%)
Body weight	30.3±1.45	28±2.3 (-8%)	30±1.15 (-1%)	27.3±3.7 (-10%)
Body weight (Reversal)	38±0.0	38±0.0 (0%)	36±0.2 (-5%)	36±0.0 (-5%)
Liver weight	2.24±0.14	2.01±0.3 (-10%)	1.93±0.12 (-14%)	1.905±0.01*** (-15%)
Liver weight (Reversal)	2.56±0.03	2.40±0.003** (6%)	2.33±0.01*** (9%)	2.323±0.001*** (9%)

In parenthesis data represents the % damage; * significant at 5% probability; ** significant at 0.01% probability; *** significant at 0.001% probability ± SEM

As compared to control, distillery soil leachate treated animals of all groups showed various histopathological alterations in the liver such as loss of radial arrangement of hepatocytes, dilation of sinusoids, cellular vacuolization, hypertrophy of the central vein, focal histocytic, focal granulomatus inflammation, binucleated hepatic cells, karyolysis, pycnosis and necrosis (fig-2.4) in comparison to liver of control mice. Besides, all above histopathological changes in 20% concentration, lytic necrosis was also observed (Figure 4).The severity of above damages in liver are directly proportional to the concentration of distillery soil leachate.

AST(45-52%), ALT(46-81%), bilirubin (62-100%) and alkaline phosphatase (17-55%) increased significantly while a decreased trend was noted for total protein (14-23%), albumin (7-26%) and globulin (14-23%) except in 5% concentration group which showed (3%) increase serum protein level in comparison to control animals (Table 2).

Tab.2. Serum biochemical parameters of distillery soil leachate treated and post treated animals.

Parameters	Control	15 days					
		5%		10%		20%	
		Treatment	Post-treatment	Treatment	Post-treatment	Treatment	Post-treatment
AST	149±1.73	216±1.16*** (45%)	209.67±4.85*** (41%)	220.67±1.2*** (48%)	211.67±1.2*** (42%)	227±1.73*** (52%)	222±1.53*** (49%)
ALT	49.33±0.49	72±1.16*** (46%)	69±0.58*** (40%)	76.33±0.88*** (55%)	71.67±1.45*** (45%)	89.33±1.45*** (81%)	83.33±2.19*** (69%)
Bilirubin	0.34±0.01	0.55±0.01*** (62%)	0.53±0.01*** (56%)	0.67±0.01*** (97%)	0.62±0.02*** (82%)	0.68±0.02*** (100%)	0.65±0.01*** (91%)
Alkaline phosphatse	74.67±0.67	115.6±1.22*** (55%)	113.33±1.20*** (52%)	101±1.16*** (35%)	98.67±0.88*** (32%)	87±1.16*** (17%)	82.33±1.45** (10%)
Protein	6.2±0.07	6.4±0.06* (3%)	5.9±0.06* (-5%)	5.33±0.15*** (-14%)	5.53±0.03*** (-11%)	4.8±0.12*** (-23%)	5.1±0.12*** (-18%)
Albumin	3.1±0.07	3.2±0.12 (3%)	2.8±0.12 (-10%)	2.89±0.01* (-7%)	2.6±0.12* (-16%)	2.3±0.17** (-26%)	2.06±0.03*** (-34%)
Globulin	3.1±0.07	3.2±0.23 (3%)	3.1±0.17 (0%)	2.44±0.01*** (-21%)	2.93±0.01 (-5%)	2.5±0.12** (-19%)	3.01±0.01 (-3%)

In parenthesis data represents the % damage; * significant at 5% probability; ** significant at .01% probability; *** significant at .001% probability ± SEM

In the reversal group (Post-treatment) recovery was not seen in any concentration group of distillery soil leachate treated animals (Figure 5, Table 2).

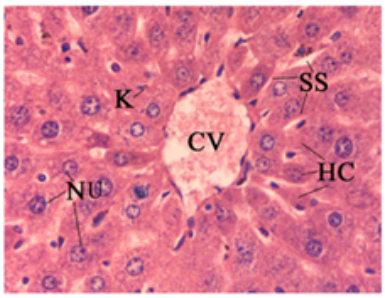

Fig. 1

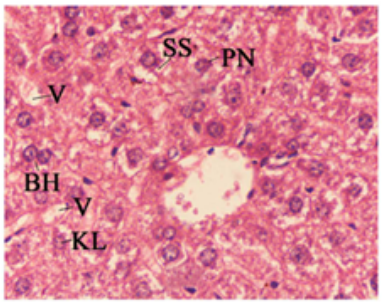

Fig. 2

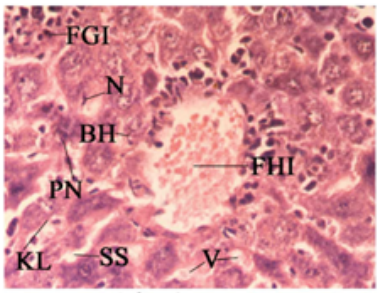

Fig. 3

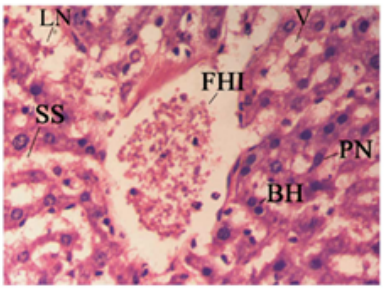

Fig. 4

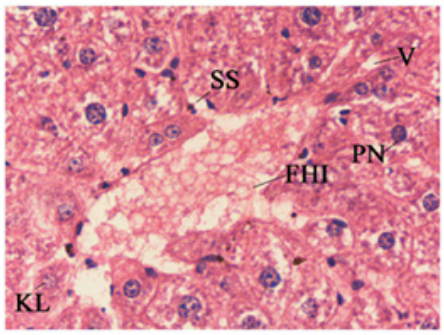

Fig. 5

Figure1 T.S. of control albino mice liver showing normal architecture of liver
Figure 2 T.S. of 5% distillery soil leachate treated mice liver
Figure 3 T.S. of 10% distillery soil leachate treated mice liver
Figure 4 T.S. of 20% distillery soil leachate treated mice liver
Figure 5 T.S. of mice liver post treatment of 5% distillery soil leachate

Discussion

Monitoring of body weight during treatment provides an index of general health status of the animals and such information may be important for the interpretation of health.

In the present study, Swiss albino mice treated with different concentrations of distillery soil leachate showed reduction in food and water intake, body weight and organ weight (Liver).Similar observations were made by other workers for these parameters [12,13,14] induced by effluents released from the textile printing industries, tanneries, pulp factories, paper mills and sugar factories in rats. The present study clearly points out that distillery soil leachate (5%, 10% and 20%) has induced histopathological changes in cellular architecture of liver (15 days). Hepatic degeneration due to the hepatic cellular vacuolation and nuclear abnormalities like karyolysis and necrosis were observed in distillery soil leachate treated mice. Similar histopathological alterations were observed by Mathur et al.[15] in liver of Swiss albino rats after treated with textile dye wastewater.
These above histopathological damages are responsible for alteration in all major functions of liver which is well proven by serum biochemical parameters.
Biochemical assays provide a relatively simple method for screening population for potential liver injury caused by occupational or environmental toxins. Liver enzymes are normally found within the cells of the liver. Increased level of AST and ALT in the blood stream indicates, that the walls of liver have been damaged and that these enzymes are leaking from the cells of the liver [16, 17].

Further, increased level of bilirubin in present investigation showed damage in kupffer cells and hepatocytes in the liver. Similar results for AST, ALT and Bilirubin was noted by Sharma et al.[17] in rats after treating with textile dye waste water .According to them increased activities of these parameters have been ascribed to their leakage from the injured hepatic parenchyma cells.

Elevated Alkaline phosphatase level was noted in present investigation which showed damage in bile duct[16].

The increase in total protein as observed in 5% (15 days) group may be due to enhanced protein synthesis to overcome the stress conditions. Increased level of protein was noted by Sharma et al.[18] in after treated with textile dye wastewater. It may be due to impairing the synthesis and metabolism of protein. Protein, albumin and globulin level decreased significantly. Depletion in the protein level has also been observed in the present communication, which may be due to impaired protein synthesis in damaged liver, altered nutritional status of the animals[19]. Thus decrease in total protein and soluble proteins indicate their metabolic utilization. Animal body contains considerable reserve of plasma protein which may be reduced by low protein diet (malnutrition). It is thus suggested that distillery soil leachate disturbs dynamic equilibrium of protein by reducing protein intake probably due to reduced food intake in treated mice as observed in the present investigation. Similar results were observed by Sharma et al. [17,18] for protein in rats after treating with textile dye waste water.

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