Efficacy of Distillery Soil Leachate on Reproductive Health of Swiss Albino Male Mice (*Mus Musculus L.*)

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Summary

The present investigation has been done to evaluate the toxic effect of different concentrations (5-20%) of distillery soil leachate on reproductive functions of Swiss albino male mice.
Body and epididymis weight decreased in all concentration groups of distillery soil leachate treated animals. Total protein content of epididymis decreased significantly (36-75%). Similar trend was observed for sperm count (78-87%) and sperm motility (20-33%) in experimental animals. Histological changed were observed in epididymis. Morphological abnormalities were also seen in sperms. Thus the accessory reproductive organ (epididymis) is important toxicological target to pollutants present in distillery soil leachate.

Key words: Swiss albino male mice; Distillery soil leachate; Epididymis, Sperm count
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Introduction

The organic effluent (Spent wash) discharge by distillery industries is one of the most complex, troublesome effluent plays major role in the environment pollution (Nagraj and Kumar [1)]. It is dark brown, acidic, having high electrical conductivity (EC). Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and Total Dissolve Solids (TDS) are several folds greater than the discharge limits as prescribed by Central Pollution control Board[2]. It is also contains heavy metals such as iron, copper, zinc and chromium(Dubey[3], Pathade[4]). Discharge of such waste directly or indirectly into environment contaminate surface water and soil as well as ground water through leaching. Recently soil leachate has been found toxic to both duckweeds and fish (Singh et al.[5]). Their toxicity however not monitored on mammals. It is therefore imperative to examine toxicity of soil leachate of such distillery waste irrigated crop fields on mammals. The epididymis provides a luminal microenvironment for sperm maturation and storage under androgen control (Robaire and Hermo[6], Robaire and Viger[7]). Hence in this communication we explored toxic effect of distillery soil leachate on Swiss albino mice with special reference to their reproductive accessory organ (epididymis and sperm morphology).

Material and Methods

Distillery soil collected from the crop field near Behrod, Alwar district (Rajasthan, India) was dried in the sun. Soil leachate prepared was considered 100%(Sharma et al.[8]), 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[9]).

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[8]. 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)

Sperm Analysis: Epididymal spermatozoa were separated as per the method of (Brooks[10]) and their counts and motility were monitored according to (Prasad et al.[11]). The smeared slides of spermatozoa stained with aqueous eosin (5%) and then observed under a microscope (10x X 40x) to assess any morphological abnormalities as described by (Feustan[12]).

Histopathology of Epididymis: Epididymis from the autopsied animals were excised out and fixed in the Bouins fixative. Sections of 5µ thickness were cut and stained (Humason[13]).

Tissue Biochemistry: Total protein content in epididymis was estimated by standard method (Lowry et al.[14]).

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 analyzing of Variance (ANOVA).

Results

In the current study, all animals survived until the end of the study period. Additionally no abnormal behavior was observed. Body weight of mice was found almost similar to control in 5% concentration, however decreased almost equal in 10% and 20% concentration group in leachate treated mice. The decreased trend was noted for epididymis weight in all concentration groups. In 20% leachate group this was almost 2 folds greater than 5% and 10% concentration groups (Table. 1).

Table.1 Body and epididymis weight of control, treated and Post- treated Swiss albino mice after exposure of distillery soil leachate at different concentrations and time interval.

		Sub-acute				Sub-acute		
Parameters	control	Treatment 15 Days			control	Post-Treatment 45 days		
		5%	10%	20%		5%	10%	20%
Body weight (gms.)	30.3±1.45	27.3±3.7 (-10)	30±1.15 (-1)	28±2.3 (-8)	38±0.0	36±1.2 (-5)	36±1.2 (-5)	38±1.2 (0)
Epididymis weight (gms)	0.058±0.01	0.051±0.01 (-12)	0.052±0.008 (-10)	0.043±0.02 (-26)	0.070	0.066 (-6)	0.068 (-3)	0.065 (-7)

Data in paranthesis indicate % change in values in comparison to control, ± SEM

The results of study also showed significant decrease in protein content (36-75%), sperm count (78-87%) and sperm motility (20-33%) in all concentration groups of distillery soil leachate treated mice (Table 2.).

Table.2 Total protein content, Sperm count, Sperm motility and Sperm abnormality of control, treated and Post- treated Swiss albino mice after exposure of distillery soil leachate at different concentrations and time interval.

		Sub-acute				Sub-acute		
Parameters	control	Treatment 15 Days			control	Post-Treatment 45 days		
		5%	10%	20%		5%	10%	20%
Protein(mg/l)	15.81±0.05	4.93±0.0.05*** (-69)	9.96±0.43*** (-36)	3.97±0.03*** (-75)	15.5±0.20	15.8±0.20 (-2)	14.47±0.23*** (-8)	8.90±0.37*** (-43)
Sperm count	59.5±4.65	9.3±2.35*** (-84%)	13.3±2.5*** (-78%)	7.75±2.45*** (-87%)	55.3±5.75	54±0.0 (-2%)	42.5±3.5*** (-23%)	43.5±0.0 (-21%)
Sperm motility (%)	77.4±6.45	33±12.37**	19.57±8.55***	30.4±22.5*	86.3±1.75	85±1.12	74.9±0.2***	66.1±0.0
Normal sperms(%)	94.78±0.74	4.12±1.07***	6.73±1.27***	5.70±2.09***	88.7±1.97	86.2±2.81	91.12±1.82	70.2±4.42***
Abnormal sperms (head abnormalities %)	4.52±1.07	72.11±2.23***	73.42±1.27***	72.03±2.02***	8.55±1.93	7.92±2.1	4.14±1.30***	15.71±2.57
Tail abnormalities(%)	0.70±0.35	24.01±1.49***	19.89±1.31***	22.26±1.77***	2.72±0.87	5.88±1.22	4.75±1.14	14.10±2.60***

Data in paranthesis indicate % change in values in comparison to control, ± SEM , Significant different at *p<0.05, ** p<0.01, *** p<0.001

Distillery soil leachate cause reproductive toxicity to Swiss albino mice which has been well proven by histopathological changes in cauda epididymis and sperm morphology (Figure 1-7 & Figure1-8).

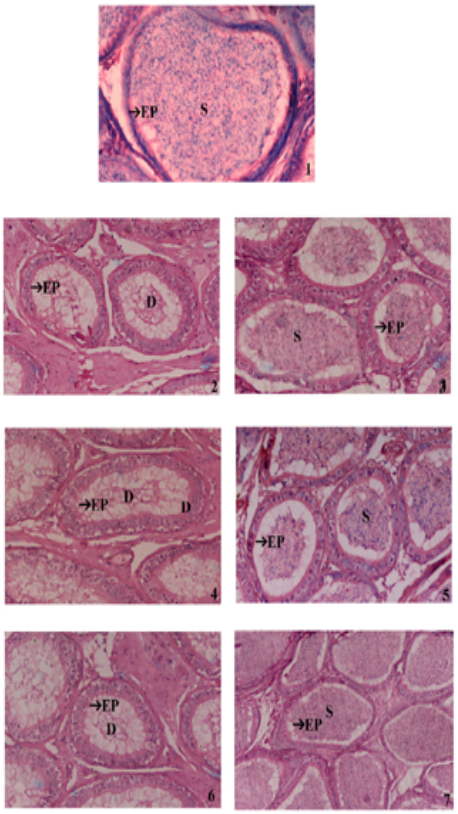

Figure1 T.S. of control albino mice cauda epididymis showing normal architecture

Figure 2 T.S. of 5 % distillery soil leachate treated mice cauda epididymis

Figure 3 T.S. of 5 % distillery soil leachate post - treated mice cauda epididymis

Figure 4 T.S. of 10 % distillery soil leachate treated mice cauda epididymis

Figure 5 T.S. of 10 % distillery soil leachate post - treated mice cauda epididymis

Figure 6 T.S. of 20 % distillery soil leachate treated mice cauda epididymis

Figure 7 T.S. of 20 % distillery soil leachate post - treated mice cauda epididymis

Plate - 2

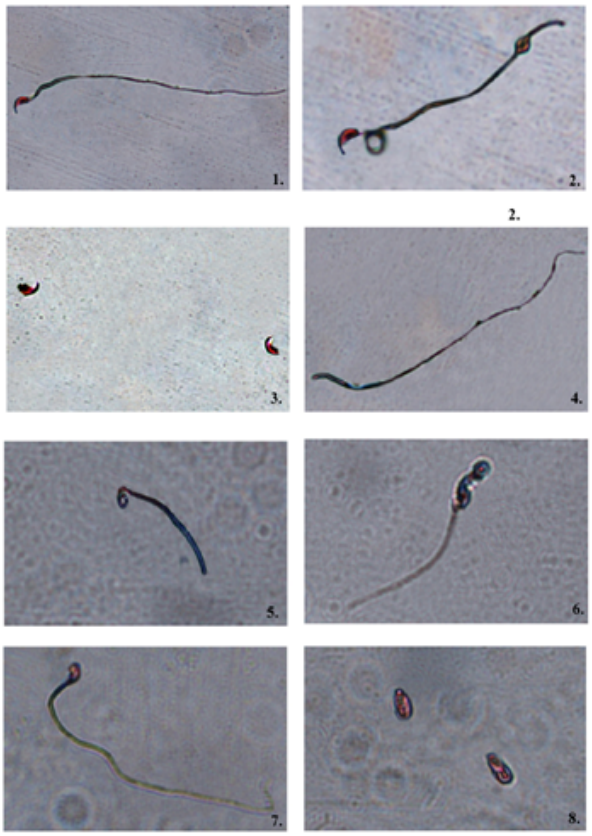

Plate 2: (Fig 1): Normal structure of sperm in Swiss albino male mice.

(Fig 2-8): Different sperm morphological abnormalities observed in head and tail region of sperms in Swiss albino male mice.

Discussion

In the present study body weight of distillery soil leachate treated animals decreased. This may be due to the presence of organic pollutants in the leachate. Similar results were observed by(Ravibabu et al.[15]) in rats after treated with industrial effluent collected from the commom effluent treatment plant located near Hyderabad. Further (Suryavathi et al.[16], Sharma et al.[17]) observed similar results in rats and mice after treated with textile dye waste water.

The weight, size and secretary functions of epididymis are regulated by androgens, it is clearly indicated,, in the study of (Agrawal et al.[18] , Choudhary et al.[19]). Further a reduction in the weight and protein content of epididymis suggested the reduced availability of androgens .

It is clear from the present data that distillery soil leachate caused significant decrease in the total protein content of cauda epididymis. The principal cells of the epididymis are responsible for the synthesis of proteins which is directly poured into the epididymal lumen (Hinton and Palladino [20], Turner et al.[21]). It is well known that the secretion of various proteins into the epididymal

lumen influences sperm maturation as it alters epididymis microenvironment, which might be a causative factor in reducing the spermcount and motility, thus reduced fertilizing ability (Verma and Chinoy[22]). Similar results were observed by (Suryavathi et al.[16], Sharma et al.[17]) in rats after treated with textile dye wastewater.

Thus, from the ongoing discussion it is clear that reduction in epididymis weight, protein content, sperm motility and count showed the toxicity of distillery soil leachate to reproductive functions of Swiss albino mice.

The histopathological alterations in epididymis and sperm morphology observed in the present study are also indicated distillery soil leachate toxicity.

This may be due to presence of organic pollutants and heavy metals in the distillery soil leachate.

Similar results were observed by (Chowdhary and Naha[23]) in male reproductive system of both exposed workers and experimental animals due to heavy metals toxicity. (Sharma et al.[24]) observed similar results in mice after treated with fluoride.

These results showed that accessory reproductive organ like epididymis is an important toxicological target of pollutants present in distillery soil leachate, as the toxicants present in distillery soil leachate alters cauda epididymal microenvironment.

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