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Pathological and Biochemical Alterations Induced by Inhalation of Furfural Vapor in Rat Lung

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Furfural is a product of hydrolysis and dehydration from raw materials containing pentosans and is widely used as an intermediate in the manufacture of plastics, agrochemicals and dyes. Furfural is considered relatively less toxic because of its low volatility (B.P. 162°C) though epidemiological data has shown that exposure of workers to 1.9-14 ppm furfural causes irritation of eyes, throat and mucous membranes, numbness of tongue, difficulty in breathing, growth retardation and hyperplasia of the olfactory epithelium (Fassett 1963).

The signs and symptoms of furfural toxicity in animals differ depending on the route of administration. Intraperitoneal administration of furfural causes a decrease in the cellular energy metabolism and an increase in lysosomal enzyme activities in the kidney (Kaminski et al. 1974), whereas, inhalation exposure resulted in biochemical changes in tissue constituents and some enzyme activities in the liver and brain of exposed animals (Ray et al. 1987; Feron et al. 1979; NIOSH 1979). Metabolic disposition of furfural and identification of its oxidative metabolites in urine of rabbits, dogs and human have been reported (Sedivec and Flek. 1978) indicating its interaction with microsomal mixed function oxidases (MFOs) which has recently been confirmed by Misra et al. (1988). The toxicity of furfural was studied because of its proposed addition to kerosene for purposes of detecting the adulteration of petrol by kerosene.

From the available literature it is apparent that little attention has been paid regarding the chain of events in the toxicokinetics of furfural, particularly in the lung, following inhalation exposure. This prompted us to undertake the systematic evaluation of the toxic response evoked in lungs of rats following exposure to furfural vapours.

MATERIALS AND METHODS

Adult male albino rats (average age 12-14 weeks; weighing 150-180 gms) of ITRC (Gheru Campus) Animal house colony, maintained under standard conditions of husbandary, apparently healthy, active and acclimatized for seven

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days prior to exposure, were used in the present study.

All the chemicals were of A.R. Grade, procurred from B.D.H. Glaxo, India and Sigma, USA. Furfural was gifted by Indian Oil Corporation Ltd., India.

The furfural vapours were generated according to the method described by Mishra et al. (1991). Six groups, consisting of 6 animals each, were exposed in an All Glass Whole Body Exposure Chamber (Dutta et al. 1988) under dynamic exposure conditions to each concentrations of furfural, viz: 1/2 LC50 i.e 95 ppm (Single exposure) and 1/5 LC50 i.e 38 ppm (repeated exposure), one hour daily, 5 days a week over the periods of 7, 15 and 30 days. The control rats were exposed to a current of compressed air only, under identical experimental condition.

Lung and liver were surgically excised and suitable portions were quickly fixed in Bouin's fluid. Sections of the fixed tissue (5 μ thick) were stained with hematoxylin and eosin for histopathological evaluation by light microscopy.

Lung of exposed animals were cleared free of extraneous material and homogenized (5% w/v) in tris buffer (0.1 mM, pH 7.2), using a Potter-Elvehjem type homogenizer for the assay of cellular constituents and enzymic activities.

The activities of acid and alkaline phosphatase, glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) were determined according to Wooton (1964). Arginase activity was estimated according to Koskowska et al. (1982). Total and non protein sulphydryls were estimated according to Jollow et al. (1974) and the protein contents in tissue preparations were measured according to Lowry et al. 1951.

The groups means and standard errors of individual observations in experimental and control group of animals were compared for evaluation of their statistical significance by student's 't' test (Snedecor and Cochran 1961). P values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Furfural exposed animals showed yellowish colouration of fur, irritation of eyes and nose, lacrimation, perinasal and perioral wetness, and respiratory difficulty. Some animals also had mild nasal bleeding and corneal opalescenee which was recovered after the termination of exposure.

Animal which died during the exposure and those sacrificed after 15 and 30 days post-exposure to furfural, exhibited enlarged and congested lung with one lobe consolidated. Such changes were not evident in case of a single exposure even at a larger dose of furfural (1/2 LC50). Liver had no gross abnormality in either group of furfural exposed animals except a little dark in colour.

Immediately after a single exposure to furfural vapours (1 hr, 1/2 LC 50), mild congestion was prevalent in the blood vessels around bronchioles and the interalveolar capillaries in lungs. Edaematous fluid was present in the perivascular areas with abundant mononuclear cells and few polymorphonuclear cells. Twenty four hours post exposure, congestion was reduced but the presence of mononuclear cells in the perivascular area was increased alongwith a few polymorphonuclear cells (Figure 1). The congestion of interalveolar capillaries was substantially higher upon repeated expsoures to furfural vapours (1 hr, 5 days a week, for four weeks) when compared to a single exposure. Presence of

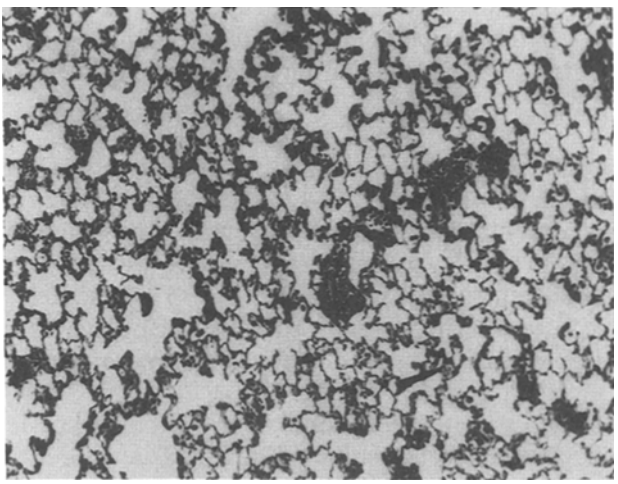

Figure 1 . Section of lungs showing the presence of mononuclear cells along with polymorphonuclear cells in the perivascular area 24 hrs post exposure to furfural (95 ppm). H & E x 120.

edaematous fluid along with mononuclear cells in the peribronchial and in the alveolar spaces was pronounced at 7 days post-exposure (Fig. 2). Congestion of interalveolar capillaries, edaematous changes and the presence of mononuclear cells were progressed further during subsequent exposure for 15 and 30 days (Fig 3).

Alkaline phosphatase activity in the lung of furfural exposed animals showed substantial elevations at all intervals upto 30 days, irrespective of a single or repeated exposure. But the acid phosphatase activity showed no significant changes in the lung (Table 1) of furfural exposed animals between 1 to 4 weeks post exposure. GPT activity increased in lungs of furfural exposed animals and was statistically significant at 2nd and 4th week post-exposure. A moderate but statistically equivocal decrease in GOT activity was observed in lung of furfural exposed rats at various time intervals upto 4 weeks. Arginase activity was consistently inhibited at all intervals over 4 weeks and the effect being more pronounced at 2nd and 4th week post exposure (Table 1).

Table 1. Effect of furfural on some clinical enzymes in lungs of albino rat.

Periods (In days)	Acid Phosphatase +	Alkaline Phosphatase +	Glutamic pyruvic Transaminase + +	Glutamic Oxaloacetate Transaminase + +	Arginase + + +	Total Sulphydryl#	Non-protein Sulphydryl#
Immediate	4.95 ± 0.25	1.82 ± 0.17	ND	5.95 ± 0.36	ND	14.73 ± 1.63	4.67 ± 1.12
	(6.17 ± 0.58)	(2.63 ± 0.13)*		(3.75 ± 0.58)*		(19.57 ± 1.38)*	(6.09 ± 0.98)
7	5.25 ± 0.29	2.32 ± 0.28	7.89 ± 0.62	5.39 ± 0.49	14.38 ± 1.18	15.13 ± 1.03	3.07 ± 0.81
	(6.77 ± 0.37)*	(3.64 ± 0.39)*	(4.11 ± 0.17)	(4.11 ± 0.27)	(13.16 ± 0.33)	(16.89 ± 0.96)	(2.63 ± 0.26)
15	4.73 ± 0.26	1.93 ± 0.31	7.36 ± 0.51	5.72 ± 0.34	15.26 ± 0.93	14.13 ± 1.21	3.73 ± 0.41
	(6.59 ± 0.40)*	(3.92 ± 0.28)*	(11.81 ± 0.64)*	(3.68 ± 0.47)*	(12.18 ± 0.64)*	(12.26 ± 0.73)	(3.43 ± 0.31)
30	4.39 ± 0.35	2.08 ± 0.34	7.36 ± 0.51	5.72 ± 0.34	14.62 ± 0.96	15.02 ± 1.92	3.29 ± 0.37
	(7.01 ± 0.17)*	(4.13 ± 0.32)*	(11.81 ± 0.64)*	(3.68 ± 0.47)*	(10.35 ± 0.35)*	(11.82 ± 0.63)	(2.07 ± 0.32)

+ = u moles phenol liberated/min/mg protein; + + = u moles pyuvate liberated/min/mg protein
+ + + = μ moles/min/mg protein; # = μg GSH equivalent/mg tissue
* = $p < 0.05$, The data are Mean ± SE for 6 observations at each intervals.
Experimental Data in parenthesis; ND = not determined.

Total sulphydryl and non-protein sulphydryl contents were significantlydecreased in lung, becoming more pronounced with the time of exposure (Table 1).

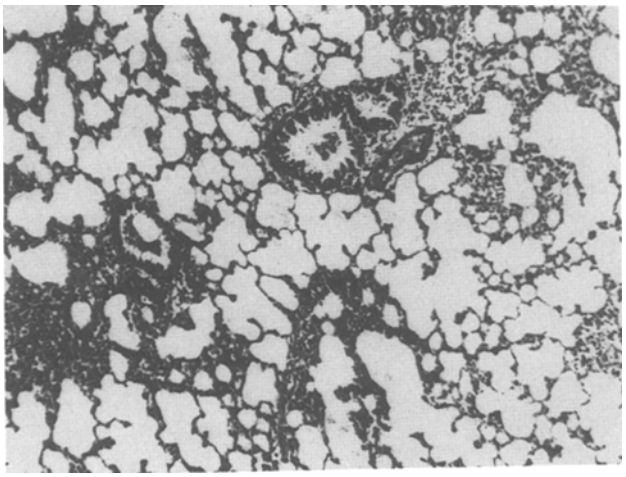

Figure 2. Section of lung showing the congestion of inter alveolar capillaries, edaematous fluid along with mononuclear cells in the peribronchial area at 7 days post exposure to furfural (1/5 L50 value, repeated exposure). H & E x 310.

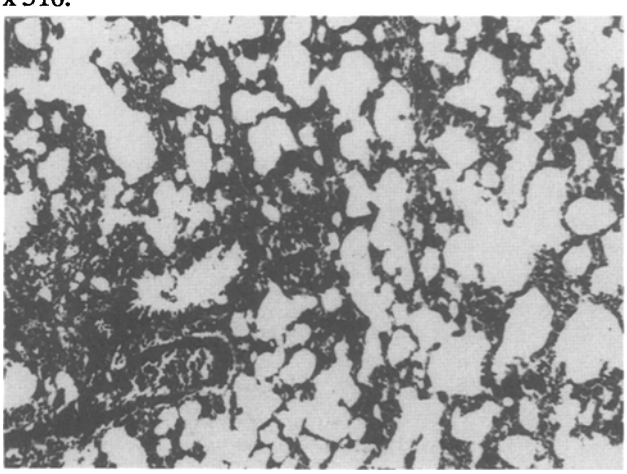

Figure 3. Section of lung showing marked congestion of inter alveolar capillaries with the presence of mononuclear cells in the perivascular and peribronchiolar areas at 30 days post exposure to furfural (1/5 LC 50 value, repeated exposure). H & E x 310.

The observed gross signs and symptoms (yellowish colouration of the fur, irritation of eyes and nose), histopathological changes (presistent edema around perivascular area) in the lungs and alteration of transaminase activity of furfural

exposed animals are in close agreement with Feron et al. (1979); Skalska-Hilgier and Szymczyk (1982) indicating furfural intoxication. Observations like hepatocyte necrosis, significant inhibition of arginase activity and elevation in acid alkaline phosphatase, GOT and GPT activities in liver of furfural exposed animals at different periods of exposure, confirms the toxic behaviour of furfural (NIOSH 1979). There was a modest decrease of total sulphydryl and glutathione (NPSH contents) in lung, even after repeated exposures for 4 weeks indicating that phase II conjugation of oxidative metabolites of furfural with sulphhydryl groups , which supports the reports of Mishra et al. (1988).

In conclusion, inhalation exposure of male rats of furfural vapours provokes pathobiochemical tissue response in the form of mucous membrane irritation, hepatocyte necrosis and hampered oxidative metabolities of furfural generated through Phase I components of MFOs. It is suggested that extended exposure to furfural can induce substantial pathobiochemical injury in biological systems.

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