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Impact of Furfural and Kerosene Co-exposure through Inhalation in Lungs of Rats

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Abstract

Furfural is being added to kerosene to check the adulteration of gasoline/high speed diesel oil. The possibility of a co-exposure of furfural and kerosene and the ability to exhibit the toxic effects of such a mixture were examined in view of the toxicity potential of the two alone and in combination with each other. A single inhalation exposure of rats to furfural was fully tolerated up to a concentration of 126 ppm. However, exposure to higher concentrations of furfural resulted in a dose dependent mortality. Exposure of rats to vapor of kerosene ranging from 426-1054 ppm did not show toxic signs and mortality up to a period of seven days. Simultaneous exposure of rats to furfural and kerosene vapors ranging in concentration from 35 ppm to 138 ppm showed a suppression of LC_{50} value of furfural. The LC_{50} was 105 ppm in rats exposed to furfural-kerosene vapors. Inhalation exposure of rats to ½ LC_{50} of furfural to 95 ppm, 1 hr daily, 5 days/week over a period of 28 days caused severe irritation of eyes and nose leading to lacrimation, perinasal and perioral wetness, labored breathing and mild nasal bleeding. Neither the body weight nor lung weight showed any change as compared to the control group. Activities of acid and alkaline phosphatase, glutamic pyruvic and glutamic oxaloacetic transaminases, succinic dehydrogenase, total sulfhydryl content and lactic acid content were evaluated.

Keywords: Furfural; Inhalation toxicology; Kerosene; Lactic acid; Succinic dehydrogenase

Introduction

The possibility of a co exposure of furfural and kerosene and the ability to exhibit the toxic effects were examined in view of the toxicity data profile available in the literature for the individual chemical entities. The toxicity data profile of kerosene has established its potential to produce skin, lung, liver and bone marrow changes in experimental animals. Likewise furfural is also known to be a moderately toxic chemical causing irritation of the eye and mucous membrane, disorders of the CNS and the biochemical injury of vital organs. It also resulted in pulmonary irritation, parenchymal injury irritation of eyes, nose, along with hyperplasia of the epithelium in the nasal cavities [1]. It was evident that a selective (cellular and/or Cytochrome-P450 isozymes specific) enhancement of pulmonary mixed function oxidases by furfural stimulates its own pulmonary biotransformation and the oxidative metabolism which was facilitated by their enzymatic conjugation with glutathione. Metabolites and excretion of ^{14}C furfural in the rat and mouse indicated presence of furoylglycine and furan acrylic acid as the major urinary metabolites. There was only subtle difference in the metabolic profile as a function of dose size and species [2]. It is anticipated that public at large may get exposed to various types of kerosene mixtures with different chemical characteristics. The effects of two chemicals given simultaneously may produce a response that may be additive, synergistic, potentiating or antagonistic to their individual responses .Therefore, toxicological potential of furfural doped kerosene mixture was evaluated in lungs of rats on simultaneous co exposure of furfural and kerosene vapors through inhalation exposure. Such studies are expected to reveal the mechanism of toxic interaction of respective chemical entities in a chemical mixture.

Methods

Chemicals

All chemicals used in the study were of analytical grade, procured from Qualigens (India) Ltd, and Sigma chemicals (USA). Furfural (2-furfuraldehyde, CAS 98:01-1) max. Purity 99% was stored in an amber color sealed container under cold and dry conditions to avoid spontaneous and oxidative decomposition during storage. White kerosene was procured from the Lucknow Railway Workshop.

Animals

Adult male albino rats (avg. age 13weeks, and body weight 165.0 ± 5.0 gm.) were maintained at ITRC (Industrial Toxicology Research Centre) animal breeding facilities under 12hrs dark / light cycles at 25°C ± 2°C and 40-60% humidity. The study was approved from Institutional Animal Ethical Committee (IAEC). All rats were housed in stainless steel wired cages and provided with pelleted feed and fresh tap water *ad libitum* throughout the period of study.

Exposure of animals

The animals were divided into four groups: consisting of six rats each.

Group I; furfural alone (F)

Group II; Kerosene alone (K)

Group III; furfural and kerosene (K+F)

Group IV; Compressed air only (C)

Group I rats were exposed to 1/2 LC_{50} (single exposure) of furfural

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essentially in the same manner as described earlier [1] using an all glass whole body exposure chamber (21 lit. capacity) under dynamic exposure condition (figure 1). The chamber concentration of furfural was 95ppm. Rats of group II were exposed to vapors of kerosene generated from 10.0 ml aliquot (426-1054ppm) using a similar set as for furfural, except that the temperature was raised to 220°C using a heating mantle in order to vaporize kerosene. The vapors were passed on to the main body of the chamber through a nebulizer. Group III rats were exposed to vapors of both, furfural and kerosene generated simultaneously. The mixed vapors were passed on through insulated glass tubes to prevent their condensation and delivered into a five-necked mixing chamber, maintained at 55°C to avoid condensation of the incoming vapors. The mixed vapors were then passed into main body of the chamber through a nebulizer along with a current of diluents air. Group IV rats were exposed to a current of compressed air only to serve as respective controls under identical specified experimental conditions. Monitoring of the chamber concentration was performed as described earlier [1] and final concentration was calculated according to NIOSH [3].

Gross observations and biochemical analysis

All rats were periodically examined for apparent signs of toxicity like irritation of eye and nose, lacrimation, external discharges / hemorrhages, general behavior, changes in fur coat and body weight and the rate of mortality. At the end of stipulated period of exposure, six rats from each group were killed by exsanguinations; lungs were surgically removed and cleaned free from arterial blood and other extraneous matter. Each lung was individually weighed and homogenized (20% w/v) in ice cold Tris buffer (0.1M, pH 7.2). The activity of Succinic Dehydrogenase (SDH) was estimated in 9000xg supernatant according to Slater and Bonner [4]. The total contents of lactic acid were estimated according to Huckabee [5] and, activities of acid and alkaline phosphatases and glutamic pyruvic and glutamic oxaloacetic transaminases by Wootton [6]. The total protein content of whole homogenate, and 9000xg fractions were estimated using Folin-Phenol reagent according to Lowry's method [7]. Glutathione content was estimated according to Jollow et al. [8].

Statistical Analysis

The group means and their standard errors for each observation in control and respective groups of exposed rats were calculated for statistical significance of results using student's t-test. P values less than 0.05 were considered significant.

Results

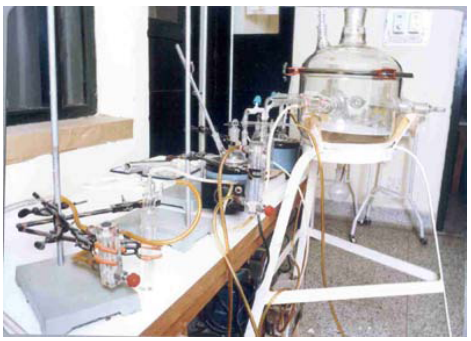

Figure 1: Inhalation assembly used for exposure of C-Compressed air only, F-furfural alone (95ppm), K-Kerosene alone (426-1054ppm), K+F-furfural and kerosene vapors (95ppm) and (426-1054ppm) respectively.

Gross observations and LC_{50} Dose

Inhalation exposure of rats to furfural vapors caused severe irritation of eyes and nose leading to lacrimation, perinasal and perioral wetness and mild nasal bleeding. There was yellowish discoloration of furfural exposed rats felt respiratory difficulty but the body weights and lung weights were not significantly altered. A few rats also had mild nasal bleeding and corneal opalescence, which was recovered shortly after termination of the exposure. A single inhalation exposure of rats to furfural was fully tolerated up to a concentration of 126 ppm; however exposure to higher concentrations resulted in dose dependant mortality leading to 100% deaths at 221ppm. Accordingly, the LC_{50} dose was found to be 189 ppm. Exposure of rats to vapors of kerosene ranging from 426-1054 ppm did not show toxic signs and mortality up to a period of 28 days. Simultaneous exposure of rats to furfural and kerosene vapors ranging in concentration from 35 ppm to 138ppm showed a suppression of LC_{50} value of furfural by 56%. The LC_{50} was 105ppm in rats exposed to furfural mixed kerosene vapors.

Autopsy

Animals that are exposed to furfural (Gr. I) and furfural kerosene mixture (Gr. III) exhibited congested lungs with one lobe heavily consolidated. Liver had no gross abnormality except a little darkening in color. Rats exposed to kerosene alone (Gr.II) did not show any abnormality.

Biochemical observation

Alkaline phosphatase activity was elevated in lungs of furfural exposed (F) and furfural and kerosene mixture exposed (K+F) rats (figure 2). The activities of acid phosphatase and Glutamic Pyruvic Transaminase (GPT) were unaltered (data not shown) in either group but that of Glutamic Oxaloacetic Transaminase (GOT) showed a significant inhibition in Furfural exposed group (F) ($P<0.01$). In Kerosene exposed group (K), however, they GOT activity was comparable to control (figure 3). Succinic Dehydrogenase (SDH) activity was inhibited in lung of furfural exposed group (F) of rats and was elevated in kerosene exposed group (K) of rats. The observed elevation was markedly suppressed in combined exposure group (K+F) of rats ($P<0.01$) though the values were still higher compared to control (figure 4). Lactic acid content in lungs of furfural exposed rats (F) showed significant increase ($P<0.001$) where as kerosene exposure resulted in depressed values. This depression was further aggravated in furfural mixed kerosene group (K+F) of rats (figure 5). Total sulfhydryls and glutathione contents did not alter significantly in either group (F), (K), or (K+F) (data not shown).

Discussion

The observed signs of furfural toxicity, viz; irritation of eyes and nose, lacrimation, external discharge, changes in fur coat etc. are in agreement with the reported findings [1]. Exposure to kerosene, on the other hand, did not reveal any gross changes of clinical significance at the reported concentrations. In close agreement to reported studies kerosene exposure alone did not result in any mortality, body weight changes and did not induce pathological changes in the lung of rats. A simultaneous exposure to vapors of furfural and kerosene in present studies resulted in a suppression of LC_{50} value of furfural (189ppm) by 56% (105ppm) which could be an indication of either a synergistic and /or antagonistic effect of individual constituents. Support for such a phenomenon was further evident from the biochemical data in present studies. It was observed that activity of oxidative enzyme, Succinic Dehydrogenase (SDH) was suppressed with a concomitant

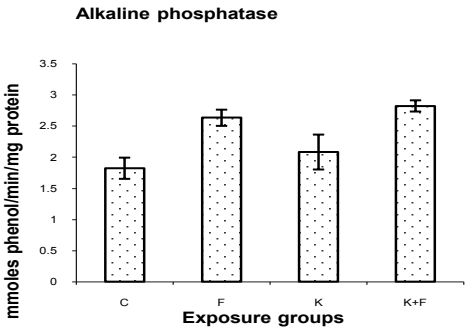

Figure 2: Alterations in alkaline phosphatase activity in lungs following inhalation exposure to furfural and kerosene vapours. The values are mean of 6 rats ± Standard Error as indicated by the bars. * *represents (p<0.001). C-Compressed air only, F-furfural alone (95ppm), K-Kerosene alone (426-1054ppm), K+F-furfural and kerosene vapors (95ppm) and (426-1054ppm) respectively.

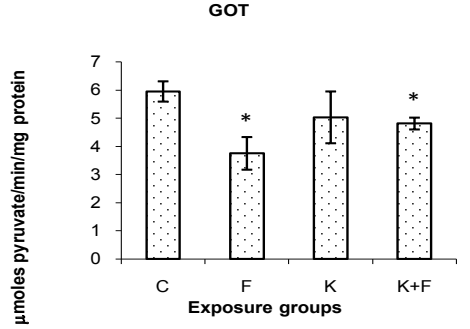

Figure 3: Alterations in glutamic oxaloacetic transaminase (GOT) activity in lungs following inhalation exposure to furfural and kerosene vapors. The values are mean of 6 rats ± Standard Error as indicated by the bars. * represents (p<0.01), C-Compressed air only, F-furfural alone (95ppm), K-Kerosene alone (426-1054ppm), K+F-furfural and kerosene vapors (95ppm) and (426-1054ppm) respectively.

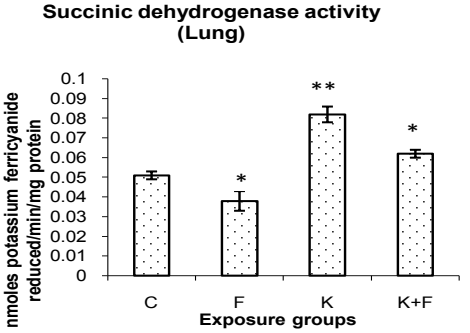

Figure 4: Alterations in succinic dehydrogenase activity in lung following inhalation exposure to furfural and kerosene vapors. The values are mean of 6 rats ± Standard Error as indicated by the bars. ** & * represents (p<0.001) & (p<0.01) respectively, C-Compressed air only, F-furfural alone (95ppm), K-Kerosene alone (426-1054ppm), K+F-furfural and kerosene vapors (95ppm) and (426-1054ppm) respectively.

increase in lactate concentration in the lung of rats exposed to vapors of furfural alone. Exposure to kerosene vapors, however, showed a reverse pattern suggesting that the two compounds may follow different pathways of metabolism and/or biotransformation [9,10]. However, on simultaneous exposure of rats to vapors of furfural and kerosene, the enhanced activity of SDH due to kerosene was suppressed with

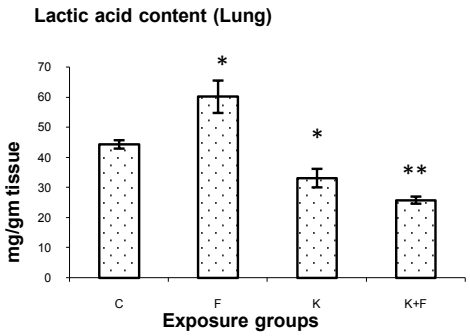

Figure 5: Alterations in lactic acid content in lungs following inhalation exposure to furfural and kerosene vapors. The values are mean of 6 rats ± Standard Error as indicated by the bars. * represents (p<.01) and ** (p<0.001), C-Compressed air only, F-furfural alone (95ppm), K-Kerosene alone (426-1054ppm), K+F-furfural and kerosene vapors (95ppm) and (426-1054ppm) respectively.

a concomitant decrease in lactate contents. A simultaneous decrease in lactate levels and an inhibition of GOT activity were also noticed. These findings are suggestive of an inhibition of cellular energy metabolism in lungs of furfural exposed rats with a simultaneous operation of non-oxidative pathways of metabolism. These findings are in agreement to those earlier reported by Danilov and Melnikova [11]. Changes in clinical enzymes viz; acid phosphatase and Glutamate Pyruvate Transaminase (GPT) in the lungs were not noticeable except mild elevations of alkaline phosphatase activity in lungs of furfural exposed rats. This is suggestive of a regenerative proliferation of type-II pneumocytes for which alkaline phosphatase is a marker enzyme [12]. Mild elevations in liver alkaline phosphatase activity may be suggestive of a possible damage to hepatocellular structure following furfural exposure. The activity was, however, unchanged in combined exposure group of rats. Ray et al. [13] had earlier shown changes in liver β-glucuronidase activity accompanied by alterations in secretory mechanism of liver as reflected by changes in serum protein pattern following s/c injection of 32 mg/kg body weight of furfural doped kerosene. The degree of lesion varies with the extent of exposure and results from toxic effects of kerosene hydrocarbons [14]. Present findings may possibly led us to explore the mode of action of individual chemical entities (furfural & kerosene) in a mixture on simultaneous exposure with particular reference to alterations in energy metabolism in the lungs using rat as an experimental model. The nature of such an interaction leading to suppression of oxidative metabolism could not be explained at the moment and needs further elucidation.

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