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SIMULTANEOUS EXPOSURE TO BENZENE AND ALCOHOL IN PETROL PUMP OPERATORS

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ABSTRACT: A study on biological monitoring of simultaneous exposure to benzene and alcohol was made in petrol pump operators of Meerut city (India). In addition to alcohol, other confounding factors viz. cigarette smoke, food habit, age and work experience were also considered. Thirty two, 20-40 years old male workers were registered for this study. Respective urine samples collected after the workshift were analyzed for specific gravity, creatinine and phenol. Phenol concentration corrected to creatinine was found to be low (22.22 mg/g creatinine) in alcoholic pump operators in comparison to non-alcoholic operators (24.09 mg/g creatinine). It has been suggested that acute ethanol ingestion inhibits benzene oxidation in the liver with an ethanol inducible form of CYP_{450}. Although urinary phenol concentration in the registered Indian petrol pump operators is less than BEI as suggested by ACGIH (50mg/g creatinine), simultaneous exposure to alcohol and benzene may potentiate the hepatotoxicity, cytotoxicity of benzene amongst petrol pump operators.

INTRODUCTION: Benzene is a petroleum constituent which affects human health. Benzene damages bone marrow & causes anemia [1]. It is a known human carcinogen that at chronic exposure poses a risk of acute myelogenous leukemia (AML) in humans [2]. Low risks of skin irritation, skin sensitization and dermatitis also exist. Its health effects include respiratory problems, kidney disease, CNS cognitive effects and effect on the reproductive system [3]. Several studies on occupational exposure to benzene have been made in different countries viz. South Korea, Thailand, China and India [4-7]. Benzene is oxidized by a microsomal cytochrome P-450 dependent monooxygenase [8].

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The principle metabolite of benzene is phenol, which is excreted via urine either in the trace form or as sulphate and/or glucuronic acid conjugates [9, 10]. However, several factors including gender, age, food habits, cigarette smoke and alcohol are known to modulate its toxicity in men and animals [11].

Ethanol is an effective inducer of hepatic drug metabolizing enzyme system. Nakajima et al studied the effects of ethanol on benzene induced hematotoxicity [12]. A synergistic effect of ethyl alcohol on the toxicity of benzene was observed by Johansson et al [13]. Any factor that influences the absorption, distribution, metabolism or excretion of a chemical can affect the result of exposure monitoring [14]. While performing biological monitoring studies in Indian petrol pump workers & traffic policemen on exposure to gasoline, it was found that cigarette smoke and food habits are potent factors that affect exposure monitoring to benzene. However, effect of alcohol consumption on biological monitoring of exposure to benzene

has not been reported. Therefore, a study on petrol pump operators consuming alcohol was made. The occupational history including alcohol consumption, smoking, food habit, age and work experience of each subject were also considered during present study.

MATERIALS AND METHODS:

Prior approval of Institutional Ethical Committee was sought to perform these investigations. Thirty two workers aged between 20-40 years, working at local petrol pump stations of Meerut City (India), were registered for this study after their consent to participate in these investigations. Similarly non occupational and healthy volunteers were also registered. Each volunteer completed a health and occupational questionnaire that included information on age, work experience, food habit, smoking and consumption of alcohol. Urine samples were collected from registered workers after the work shift. Samples were collected in white sterilized plastic bottles and transported in an ice box to the laboratory and stored at -20^{0}C for further analyses-

1. Specific Gravity:

Specific gravity of urine samples was determined using an urinometer (Atago Company Ltd, Tokyo, Japan). Samples with specific gravity ranging from 1.010 to 1.030 only were selected for further analysis.

2. Creatinine:

Creatinine in urine samples was estimated using a commercial kit obtained from Span Diagnostics (India) through a method suggested by Toro and Ackerman[15].

3. Determination of phenol:

Phenol in urine samples was determined by the method of Yamaguchi and Hayashi[16]. Assay was based on the condensation reaction with 4-aminoantipyrine which oxidized with alkaline ferricyanide to give a red complex. The absorbance was recorded at 500nm using a spectrophotometer (Systronic, India). Pure liquid phenol (Merck Ltd., India) was used as the standard.

Statistical inferences:

Statistical data was evaluated by student's "t" test.

RESULTS:

Results obtained during present investigation are presented through tables 1-4. Only those samples, which showed specific gravity between 1.010 to 1.030 were considered for analysis of creatinine and phenol. The study was made amongst alcoholics and non-alcoholics, smokers and non-smokers, vegetarians and non-vegetarians. In addition, effect of age and work experience was also studied.

Creatinine is a reliable marker of renal function. Cigarette smoke and alcohol consumption both were found to affect creatinine values amongst petrol pump workers. Alcohol consumption resulted into higher creatinine excretion than smoking. However, alcoholics and smokers excreted less creatinine than non- alcoholics and smokers. Smoking and alcohol both were found to exert confounding effects on metabolism of benzene in petrol pump workers. This was witnessed by results on alcoholics and smokers. Higher values were obtained in non alcoholics and smokers in comparison to alcoholics and smokers. Further, values for phenol were found to be higher in non alcoholics than alcoholic workers **(Table 1)**.

TABLE 1: EFFECT OF SMOKING ON CREATININE AND PHENOL IN ALCOHOLIC PETROL PUMP OPERATORS.

Groups	Creatinine (mg/L)		Phenol (mg/g creatinine)	
	Petrol pump operators	Non-occupational volunteers	Petrol pump operators	Non-occupational volunteers
Alcoholics (Smokers)	2.59±0.94*	3.65±0.53	22.22±0.09*	6.63±0.18
Non-alcoholics (Smokers)	2.03±0.24^{NS}	1.43±0.11	24.09±0.10*	19.59±0.53
Alcoholics (non-smokers)	5.35±0.39*	2.50±0.00003	7.82±0.04^{NS}	7.89±0.07
Non-alcoholics (non-smokers)	3.92±0.20*	1.80±0.56	12.86±0.73*	6.06±0.02

Values are expressed as mean ± SE, (n=5)

*****Denotes values are significantly different from non-occupational volunteers ($p<0.05$)

^{NS} Denotes non significant differences

Type of food also influenced the metabolism of benzene in petrol pump operators. Vegetarians and non alcoholics excreted less creatinine in comparison to alcoholics and vegetarians. Alcoholics and non-vegetarians excreted less creatinine than non- alcoholics and non vegetarians. Non-vegetarian food together with alcohol accelerated metabolism of benzene in comparison to non alcoholics and non vegetarians. Vegetarian food and alcoholics did not have significant effect on benzene metabolism **(Table 2).**

TABLE 2: EFFECT OF FOOD HABIT ON CREATININE AND PHENOL IN ALCOHOLIC PETROL PUMP OPERATORS.

Groups	Creatinine (mg/L)		Phenol (mg/g creatinine)	
	Petrol pump operators	Non-occupational volunteers	Petrol pump operators	Non-occupational volunteers
Alcoholics (Vegetarians)	3.42±1.1*	2.65±0.87	13.66±0.44^{NS}	12.92±0.06
Non-alcoholics (vegetarians)	2.58±0.37^{NS}	2.13±0.21	12.97±0.05*	8.91±0.02
Alcoholics (non-vegetarians)	1.92±0.5*	3.84±0.56	26.48±0.22*	6.01±0.04
Non-alcoholics (non-vegetarians)	2.01±1.6^{NS}	2.46±1.7	18.83±0.14*	6.02±0.04

Values are expressed as mean ± SE, (n=5)
*****Denotes values are significantly different from non-occupational volunteers ($p<0.05$)
^{NS} Denotes non significant differences

Cohort included petrol pump operators engaged in the job from 1 to 15 years. All consumed alcohol. Those possessing minimum experience of 1-5 years excreted more creatinine than those having the job experience of 6 to 10 years. Minimum values were recorded amongst those engaged for 11-15 years. Duration of exposure to benzene had confounding effect on its metabolism. It was lowest in workers having 1-5 years of job experience. Highest values were recorded for those exposed to benzene vapour for 11-15 years **(Table 3)**.

TABLE 3: EFFECT OF WORK EXPERIENCE ON CREATININE AND PHENOL IN ALCOHOLIC PETROL PUMP OPERATORS

Duration of work (years)	Creatinine (mg/L)	Phenol (mg/g creatinine)
Alcoholics (1-5 years)	3.92±1.57*	10.36±0.52^{NS}
Alcoholics (6-10 years)	3.06±0.54^{NS}	14.11±0.16*
Alcoholics (11-15 years)	2.00± 0.064^{NS}	30.02±0.15*
Non-alcoholics(1-5 years)	2.62±1.5	10.16±0.19

Values are expressed as mean ± SE, (n=5)
*****Denotes values are significantly different from non-alcoholics ($p<0.05$)
^{NS} Denotes non significant differences

This study comprised workers ranging in age from 20 to 40 years. Those in the age group of 20 to 30 years excreted less creatinine than those in the age group of 30-40 years. Non- alcoholic workers excreted less creatinine in comparison to alcoholics. Age also exhibited pronounced effect on the metabolism of benzene in alcoholic petrol pump workers. Phenol concentration in urine was higher in the workers of age group of 20-30 years in comparison to those in the age group of 31-40 years **(Table 4)**.

TABLE 4: EFFECTS OF AGE ON CREATININE AND PHENOL IN ALCOHOLIC PETROL PUMP OPERATORS.

Groups	Creatinine (mg/L)		Phenol (mg/g creatinine)	
	Petrol pump operators	Non- occupational volunteers	Petrol pump operators	Non- occupational volunteers
Alcoholics (20-30 years)	2.74±1.02^{NS}	2.26±1.1	15.57±0.16*	13.01±0.24
Alcoholics (31-40 years)	3.02±0.43^{NS}	3.75±0.80	13.99±0.04*	6.16±0.07
Non-Alcoholics (20- 30 years)	1.87± 1.23^{NS}	2.08± 2.6	15.00±0.13*	8.13±0.11

Values are expressed as mean ± SE, (n=5)
*****Denotes values are significantly different from non-occupational volunteers ($p<0.05$)
^{NS} Denotes non significant differences

DISCUSSION: Petrol pump operators are occupationally exposed to benzene vapour. These operators come from different ethnic background. They possess different food habits. Many of them either smoke or consume alcohol or addicted to both the habits. Amongst these habits, alcohol intake has been a predominent factor. Therefore, a very important question that needs to be addressed is whether alcohol consumption has any influence on the susceptibility of petrol pump operators to noxious effects of benzene.

It has been reported in the literature that chronic ethanol consumption significantly enhanced the metabolism of a variety of volatile hydrocarbons in rat liver [17]. Ethanol has been known to potentiate the toxicity of some hepatotoxic agents such as carbontetrachloride and chloroform [18]. An underlying mechanism proposed for this potentiation is that ethanol induces enzymes that accelerate the biotransformation of these hydrocarbons to highly reactive intermediates [19]. Phenol, being the major metabolite of benzene has been treated as the biomarker of benzene exposure[20]. Present results on phenol are thus indicative of occupational exposure to benzene.

It was observed that values for phenol were higher in non-alcoholic workers. Acute ethanol ingestion inhibits benzene oxidation in the liver with an ethanol-inducible form of CYP 450 [13]. In contrast with the stimulating effect of chronic ethanol consumption, acute ethanol intake inhibits the metabolism of several xenobiotics [21]. Recently, effects of simultaneous exposure to benzene and ethanol on benzene metabolism in mice were investigated by Mikov et al [22]. Urinary thioether excretion significantly decreased in the mice receiving both benzene and alcohol compared to mice receiving benzene only. In another study made by Marrubini et al [23] in mice, it was concluded that ethanol exacerbates benzene myelotoxicity but reduces the urinary excretion of benzene metabolites in urine, suggesting that the influence of ethanol intake should be considered carefully in biomonitoring of benzene exposure.

In addition to alcohol, other confounding factors like smoking were also investigated. Non-smokers and alcoholics excreted less phenol than smokers and alcoholics. Blood benzene concentration has been found to increase significantly amongst smokers in occupationally exposed individuals and amongst the control group [24]. Effect of food (vegetarian/non-vegetarian) in alcoholic petrol pump operators was also studied. Non-vegetarian food together with alcohol accelerated the metabolism of benzene. Phenol in urine was higher in workers of age group of 20-30 years in comparison to those in the age group of 31-40 years. An exponential increase in excretion of phenol was observed with the increase in duration of exposure to benzene. The metabolism of xenobiotics is known to be modulated by social habits [25].

These observations are supported by the fact that both benzene and ethanol compete for CYP2E1 which is required for the metabolism of benzene and ethanol [26]. Although, urinary phenol concentration in Indian petrol pump workers is less than biological exposure index (BEI) as suggested by ACGIH (50 mg/g creatinine) [27], simultaneous exposure to benzene and alcohol may potentiate the hepatotoxicity, cytotoxicity and genotoxicity amongst workers occupationally exposed to benzene.

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