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Effects of endocrine disrupting chemicals from leather industry effluents on male reproductive system

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ABSTRACT

The leather tanning industry is characterized by the production of different kinds of effluents, generated in each step of leather processing. These effluents have various chemical compounds which may cause toxicity and endocrine disruption and are thus known as endocrine disrupting chemicals (EDC). This study was aimed to examine the androgenic potential of leather industry effluents collected from northern region of India. Hershberger assay data showed a significant increase ($p < 0.05$) in the weight and structure of sex accessory tissues of castrated rats. Reverse transcriptase polymerase chain reaction (RT-PCR) analysis demonstrated a significant change ($p < 0.05$) in the expression patterns of the major steroidogenic enzymes in adrenal and testes namely, cytochrome P450scc, 3β-hydroxysteroid dehydrogenase, 17β-hydroxysteroid dehydorgenase in castrated and intact rats. This was further supported by increased enzymatic activities measured *in vitro* spectrophotometrically. Serum hormone profile demonstrated a dose dependent increase in testicular and adrenal testosterone productions in intact and castrated rats, respectively. This was further supported by decreased level of gonadotrophic hormones (LH and FSH) in treated groups of animals. Further, the effluent treatment resulted in the development of hyperplasia in seminiferous tubules of testes in treated rats as evident from histopathological studies and about two-fold increases in daily sperm production. On analysis of water samples using GC–MS, it was found to contain various aromatic compounds (nonylphenol, hexaclrobenzene and several azo dyes) some of which independently demonstrated similar effects as shown by water samples. Our data suggests that the effluents from leather industry have potential EDC demonstrating androgenic activities.

1. Introduction

There is increasing scientific evidence that many substances with different chemical structures can interfere with the normal hormonally regulated biological process to adversely affect the development and/or reproductive function in wildlife, experimental animals, and humans [1]. These environmental contaminants are able to alter the normal functioning of the endocrine and reproductive systems by mimicking or inhibiting endogenous hormone actions, or modulating the synthesis of hormones [2]. These types of chemicals have been given the term "endocrine disrupting chemicals" (EDC). These EDC are composed of various types of chemicals used in a wide variety of herbicides, fungicides, insecticides, detergents, material of plastics and various industrial effluent contaminants. Although the concentration necessary to disrupt endocrine regulation may be lower than the carcinogenic level, yet the life long intake of even very low levels of these compounds may disturb the delicate hormone balance and compromise the reproductive fitness and health of many species and ultimately may lead to carcinogenesis [3]. More importantly, EDC may pose species-specific risks that are difficult to investigate because they also often act silently with severe latent adverse effects [4,5].

A large pool of literature exists for EDC with estrogenic potential and a number of environmental hazards with estrogenic properties have been identified and classified [6]. Although, there are similar health concerns regarding androgenic EDC that can reduce sperm production, alter genital development, various types of cancer, cryptorchadism, decreased male reproductive capacity and contribute to neurological syndromes in males, yet the identification and classification of these putative health hazards have progressed slowly. Recent reports of several non-steroidal compounds that have the ability to bind and activate the androgen receptor (AR) are of particular concern because many of these xenobiotics are ubiquitous in daily life and some are manufactured and/or are released out as industrial effluents [5,7].

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Effluents of leather industry are of great concern to agencies responsible for environmental management. Some of the authorities consider it to be one of the 10 most harmful industrial effluents to the environment, responsible for extreme pollution of water resources and generating substances leading to deterioration and death of wide range of organisms [8,9]. Several chemicals are used in various steps of leather processing at different time period giving leather effluent a very composite nature. Effluents of leather industry have been reported to contain formaldehydes, chromates and bichromate salts, aniline, benzene based dyes, other different organic chemicals (butyl acetate, ethanol, benzene, toluene, nonylphenols, polychlorinated phenols), dimethyl formamide, sulphuric acid, ammonia, hydrogen sulphide and others [10,11]. A number of studies have shown that these chemicals cause endocrine disruption and have other harmful effects on development and reproduction of animals. However, in majority of cases only physical and chemical tests have been used to evaluate the impact of these industrial effluents. These tests satisfy the criteria set by state agencies for environmental control, but several authors have recommended that physical and chemical analyses with tests using organisms be utilized to evaluate toxicity of the compounds resulting from anthropogenic activity [12,13]. Whatever data obtained till date on the endocrine disrupting nature of leather industry effluents, they give a superficial idea about their toxic and disrupting effects. To the best of our knowledge there are no other studies to understand the role of leather industry effluents as a source of androgenic contaminants using animal models. This is critical from the Indian environmental scenario since leather industry constitutes one of the major industries in India.

The aim of this study was to understand the molecular mechanism of action of androgenic EDC of the leather industry effluents. Water samples were collected from a common site receiving leather effluents from more than 100 small and big leather industrial units in Northern part of India. Here, an integrated approach was adopted, first, screening the samples for their androgenicity by Hershberger assay and when samples were found positive for androgenicity, molecular mode of action of probable EDC was studied by various parameters after gavaging the samples to intact rats. Finally the contaminating chemicals were identified, quantified and tested in animals to get a holistic mode of action of the chemicals. Since during leather processing various chemicals are used at different times so samples were collected from a site which was supposed to represent the chemicals involved in almost all steps of leather processing.

2. Materials and methods

2.1. Sample preparation

Samples were collected monthly from May to November, 2006, in amber colored glass bottles, rinsed initially with acetone and MilliQ water (Millipore, India) from the site receiving effluent from a number of leather industrial units and stored at 4 °C. The total samples collected during this period were pooled to reduce the chances of variations arising during sampling. All the glass wares to be used in the experiment were rinsed with dichloromethane (DCM) with 0.6% concentrated hydrochloric acid to prevent clinging of the steroids with glass walls. Collected water samples were aliquoted into three parts viz. 18, 36 and 72 l. All sample aliquots were filtered and were subjected to organic phase extraction by adding DCM according to the method described earlier [14] with slight variations as per our laboratory conditions. DCM was added to each aliquot at the ratio of 60 ml/l of crude sample and mixed thoroughly for 2 min, left for 10 min for settling down of organic phase and finally the aqueous phase was separated by separating funnel. The procedure was repeated thrice to extract organic phase and all aqueous phase was poured off. Extracted organic phases were mixed and concentrated under reduced pressure on a Buchi rotatory evaporator to 2 ml/l of crude sample and concentrated organic phase was solvent exchanged with 10% ethanol. At the final preparation, single dose per animal consisted of 150 μl of prepared sample and this single dose per animal was equivalent to 300, 150, and 75 ml for 72, 36 and 18 l of crude water samples, respectively. Prepared dose was stored at −20 °C until gavaged to the animals.

2.2. Animals

Study was done on the male albino rats, *Rattus norvegicus,* 8 weeks of age, with the approval of institutional ethical committee. Animals were purchased from the animal house facility of All India Institute of Medical Sciences (New Delhi, India) and were in healthy condition at the time of purchasing. They were housed in a well-ventilated animal house with 12 h light:12 h dark schedule. The animals were fed with a balanced animal feed (Ashirwad Animal Feed Industries, Punjab, India) and had access to normal drinking water at all the times. The animals were acclimatized to the animal house condition for 10 days prior to the experiments. All the procedures were approved by the Institutional Animal Ethics Committee and conformed to the UFAW Handbook on the Care and Management of Laboratory Animals.

2.3. Experimental design

Study was done in two parts—first part dealt with Hershberger assay to screen the test sample for their androgenicity and second part dealt with analyzing the mode of endocrine disruption of leather industry effluents using intact animals.

2.3.1. Screening of test samples for androgenicity

Rats were castrated by removing testis and epididymis and allowed to recover for next 10 days. The animals were then grouped ($n = 6$) as follows:

- Group I: treated with only alcohol (10%) as vehicle
- Group II: treated with 75 ml equivalent of water samples
- Group III: treated with 150 ml equivalent of water samples
- Group IV: treated with 300 ml equivalent of water samples

Vehicle and extracted water samples were administered via gavages to the castrated rats for 20 consecutive days. The used dose in the experiment was optimized earlier and was below the LD_{50} concentration. Approximately after 24 h of final treatment, androgen-dependent accessory sex organs (SATs) namely, ventral prostrate, seminal vesicles, glans penis, vas defferentia, Cowper's gland were carefully removed and weighed.

2.3.2. Intact assay

Intact rats were grouped ($n = 6$) as follows:

- Group I: treated with only alcohol (10%) as vehicle
- Group II: treated with 75 ml equivalent of water samples
- Group III: treated with 150 ml equivalent of water samples
- Group IV: treated with 300 ml equivalent of water samples
- Group VI: treated with 4-aminobiphenyl at a concentration of 2.0 μg/l
- Group V: treated with hexacholrobenzene at a concentration of 3.0 μg/l