List of Publications


Micronuclei induction and chromosomal aberrations in *Rattus norvegicus* by chloroacetic acid and chlorobenzene

Mohammad Faisal Siddiqui, Riaz Ahmad*, Waseem Ahmad, Absar-ul Hasnain

Section of Genetics, Department of Zoology, Aligarh Muslim University, Aligarh 202002, Uttar Pradesh, India

Received 17 August 2005; received in revised form 2 March 2006; accepted 4 March 2006

Available online 2 May 2006

Abstract

Chloroacetic acid (CAA) and chlorobenzene (CB) have been evaluated for in vivo mutagenic potential in *Rattus norvegicus*, employing the following criteria: (i) chromosomal aberrations (CAs) such as breaks, gaps, exchanges, rings, and multiple aberrations and (ii) micronuclei (MN) induction. Three sublethal doses, 0.008, 0.01, and 0.012 mg/g b. wt. of CAA and 0.75, 1.0, and 1.25 mg/g b. wt. of rat of CB were administered and the bone marrow cells evaluated in each of the three treated groups at 12, 24, and 48 h, respectively. Mean MN frequencies of 4.40 ± 0.2 and 5.42 ± 0.3, obtained respectively for CAA and CB. The higher induction of MN by CAA and CB was dose- and time-dependent. Most significant impact (P < 0.05) for either of the compounds was observed at 24 h post administration, when the recorded mean frequency of CAs was maximum for CAA (4.33 ± 0.6) as well as for CB (4.66 ± 0.5).

2006 Elsevier Inc. All rights reserved.

Keywords: Micronuclei induction; Chromosomal aberrations; Chloroacetic acid; Chlorobenzene; *Rattus norvegicus*; Mutagenic potential

1. Introduction

Several studies were conducted on mammals to demonstrate the mutagenic effects of chemical compounds by including the chromosomal abnormalities as the parameter of mutagenic potential (Moore et al., 1996; Sharma et al., 2000; Swada et al., 1987). These reports indicate that some routinely used chemical compounds may be mutagenic to human population suggesting the ever increasing risks of human exposure to hazardous chemicals (Colacci et al., 1991; Quick et al., 1983; Saghiri et al., 2001; Sharma et al., 2000; Swada et al., 1987). Studies conducted on farmers and industrial workers have indicated that the exposure to such hazardous compounds causes both somatic and hereditary mutations (Antonelli et al., 2003; Farah et al., 2003; Major et al., 1993; Schmid et al., 1982). Work on in vivo mutagenic potential of chemical compounds on mammals, by including the chromosomal aberrations (CAs) in particular, is still scarce. In this report, we have evaluated the in vivo mutagenic potential of chloroacetic acid (CAA) and chlorobenzene (CB) in adult male *Rattus norvegicus* by employing (i) CAs such as breaks, gaps, exchanges, rings, translocations, and multiple aberrations and (ii) micronuclei (MN) induction following the intra peritoneal administration of the sublethal doses of CAA and CB. So far as we know, the two compounds have not been studied in a mammalian system simultaneously.

CAA is a halogenated aliphatic carboxylic acid that is widely used as herbicide and preservative for curing hay. It is one of the most commonly detected disinfectant byproducts in the drinking water supply of the United States (Christman et al., 1983; Plewa et al., 2002). Considerable information is available on CAA describing its mutagenic effects in humans and other mammals where it is reported to penetrate skin and cause serious tissue as well as cytological damages (Kulling et al., 1992; Kusch et al., 1990; Quick et al., 1983). Its kinetics after intravenous injections at subtoxic and toxic doses have been worked out in adult-male rats by Saghiri et al. (2001).

The other chemical of this study is a chlorinated benzene compound, CB. It is used in the synthesis of organochlorine pesticides such as DDT, phenol, and picric acid and as a chemical intermediate in the synthesis of nitrochlorobenzenes and in the manufacture of dyes and perfumes (U.S. EPA, 2003; Vaghef and Hellman, 1995).
ADJUSTMENTS OF SERUM LACTATE DEHYDROGENASE ISOENZYMES AND THEIR SIGNIFICANCE IN MONITORING THE TREATMENT IN PATIENTS WITH TUBERCULAR PYOTHORAX

Riaz Ahmad, Mumtaz Alam*, M Faisal Siddiqui and Absar-ul Hasnain

Section of Genetics, Department of Zoology, Faculty of Life Sciences, AMU, Aligarh-02 (UP) INDIA
*Department of Surgery, Jawaharlal Nehru Medical College and Hospital, AMU, Aligarh-02 (UP) INDIA

ABSTRACT
The adjustments and diagnostic significance of polyacrylamide gel electrophoretic (PAGE) profiles of lactate dehydrogenase isoenzymes (LDH: 1.1.1.27) was evaluated in the sera and pleural fluid of patients with tubercular pyothorax. Sera and pleural fluid samples were randomly collected from 72 and 18 patients respectively at two different timings; first, when patients were admitted to the Hospital and second, after an intensive phase of treatment. Sera of 20 healthy individuals served as control. Our results demonstrate significant differences in sera LDH (sLDH) and pleural fluid LDH (pLDH) isoenzymes. In patients the order of LDH isoenzyme in sera and pleural fluid followed: LDH-5>-4>-2>-3>-1 and LDH-5>-4>-3>-2>-1 respectively. The ranking of activity levels in control was LDH-2>-1>-3>-5>-4. In the second phase of sampling from 31 patients, values of sLDH isoenzymes showed recovery and resembled profiles of controls. Therefore, the sLDH zymograms of patients can be used as the prognostic marker, since they tend to reach the normal level during recovery signifying the effect of chemotherapy in hospitalized patients. Moreover, according to the present findings on LDH-PAGE profiles, the levels of LDH-5 and -4 rise in pyothorax patients significantly (P<0.05). This elevation along with the rise in total LDH activity may, therefore, be used in the diagnosis and monitoring of tubercular pyothorax.

KEY WORDS
Lactate dehydrogenase, Sera LDH profiles, Polyacrylamide gel electrophoresis patterns, Pleural effusion, Tubercular pyothorax.

INTRODUCTION
Pyothorax is one of the common pleural diseases in which collected pus in pleural space limits lung expansion. As a result, toxins released into the blood stream causes further stress finally ending in empyema. The pathological description of empyema includes the combination of a thick cortex of fibrin and coagulum over the lung. Etiology of pleural effusions is quite varied (1-3). Pleural effusions are classified as either transudates or exudates based on fluid protein and lactate dehydrogenase (LDH) concentration. Transudative effusion occurs as the result of a change in fluid balance in the pleural space. An effusion is considered exudative if it meets any one of the criteria described elsewhere (4). Lymphomas are an important cause of malignant effusion and account for 10% to 14% of all malignant pleural effusions. Pyothorax-associated lymphoma (PAL), a non-Hodgkin’s lymphoma is a clinical complication consequent to a long-standing history of pyothorax develops in the pleural cavity (5). A few studies have been carried out where the correlation of interleukin-6 (IL-6) or interleukin-1 beta (IL-1 beta) concentrations in the pleural fluid of pyothorax patients with its pathophysiology has been demonstrated. Some workers have compared common biochemical parameters such as cholesterol, total protein and albumin (6-7).

Clinically, a number of enzymes (or isoenzymes) have for long been recognized for their value as biochemical markers (6,8,11,14-15,20). One such enzyme is Lactate
Effects of an aqueous extract of *Croton bonplandianum* Baill in rats

Riaz Ahmad\(^a\), Abdul V. Khan\(^b\), Mohammad F. Siddiqui\(^c\), Absar-ul Hasnain\(^d\)*

\(^a\) Section of Genetics, Department of Zoology, Faculty of Life Sciences, Aligarh Muslim University, Aligarh, 202002 Uttar Pradesh, India
\(^b\) Department of Botany, Faculty of Life Sciences, Aligarh Muslim University, Aligarh, 202002 Uttar Pradesh, India
\(^c\) Interdisciplinary Brain Research Center, J.N.M. College and Hospital, Aligarh Muslim University, Aligarh, 202002 Uttar Pradesh, India

**ABSTRACT**

We have investigated the cytotoxic and biochemical effects of injecting aqueous phytoextract of *Croton bonplandianum* (Baill) leaves in male rats. Subchronic dosages of 3.25, 4.65 and 6.97 mg phytoextract g\(^{-1}\) b.wt. week\(^{-1}\) were administered to rats. To test ameliorative effects, rats were injected with phytoextract mixed with 0.2 mg g\(^{-1}\) b.wt. of cyclophosphamide. Positive controls received only cyclophosphamide, while negative control groups were kept on normal diet and water. Our results demonstrate that phytoextract did not induce micronuclei formation in rats and shows insignificant amelioration (P < 0.05). However, differences in serum LDH isoenzymes, ALP, SCOT, SGPT activities and bilirubin were remarkable and displayed dose as well as duration dependent variations. The most outstanding observation of this study was the release of cardiac Tnl in sera of rats injected with 6.97 mg g\(^{-1}\) b.wt. of phytoextract for 21 days. Our findings suggest that at the highest concentrations used here phytoextract of *C. bonplandianum* is not clastogenic; instead it is cardio- and hepatotoxic.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

According to an estimate, 80% of the world population in developing countries relies on traditional plant medicine (Salatino et al., 2007). Analyses of these plant extracts or their active ingredients by modern laboratory techniques have generated renewed interest in their medicinal importance. Our study deals with the cytotoxic and biochemical effects of a medicinal plant *Croton bonplandianum* Baill on rats, *Rattus norvegicus*. This *Croton* species was introduced to India during the late 1890s from Paraguay. Within 50 years, its distribution became countrywide. For decades, chemicals and products derived from *Croton* species and their phyto-derivatives have been used to cure human ailments and several published reports reaffirm their medicinal value (Carvalho et al., 1996; Brito et al., 1998; Campos et al. 2002; Guerrero et al., 2002). A few of these reports had specific focus on genetic or mutagenic/clastogenic effects on vertebrate systems that included micronuclei counts also (Agner et al., 1999, 2001; Santos et al., 2006b). However, the above cited published evidence mostly dealt with *Croton* species other than *C. bonplandianum* (family Euphorbiaceae). *C. bonplandianum* is also a plant of considerable medicinal and agricultural importance (Datta and Sinha-Roy, 1975; Jain, 1991; Asolkar et al., 1992; Kumar et al., 1997). Its latex and leaf extract (phytoextract) have previously been reported to help in healing cuts or open wounds and external treatment for ringworm infection (Jain, 1991). N-Me crotsparine, a chemical constituent of *C. bonplandianum* leaves had significant hypotensive effect on cats (Asolkar et al., 1992).

Although the induction of micronuclei by crotus leaves has previously been reported (Santos et al., 2006b), there exists no report on the changes in lactate dehydrogenase isoenzymes. Literature on quantitative variations in total LDH activity (TLDH) as diagnostic markers of various human diseases is, however, extensive (Hammond et al., 1975; Rotenberg et al., 1988; Jaffe et al., 1996). By electrophoresis, lactate dehydrogenase (NADH ↔ NAD oxidoreductase) typically resolves into five isoenzyme bands. Homotetramer isoenzyme LDH-5 predominates muscle while LDH-1, the heart. Random tetramerization of the two constituent subunits results in the formation of three heterotetramers LDH-2, LDH-3 and LDH-4. We have recently demonstrated that adjustments in the levels of various LDH tetramers may be a sensitive marker of some clinical conditions (Ahmad et al., 2008). Despite medicinal importance, effect of *Croton* phytoextracts on cardiovascular system was seldom investigated (Guerrero et al., 2002, 2004; Silva et al., 2005). Using cTnl immuno-cross reactivity, we obtained conclusive evidence of the cardiac damage by *C. bonplandianum* leaves extract.

The present investigations were planned keeping in mind the paucity of therapeutic information on *C. bonplandianum* (Baill) reviewed above. The effects of injecting subchronic doses of *C. bonplandianum* leaves extracts (phytoextract) were monitored in *R. norvegicus*. We preferred aqueous extracts, because in Indian subcontinent traditional medication is based on administration in this form, while intraperitoneal route was envisaged to produce