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Experimental assessment of arsenic toxicity in garole sheep in India

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ABSTRACT

Arsenic, a dangerous bio-accumulative poison, is a grave threat affecting a large number of people as well as animals throughout the World, particularly in Bangladesh and West Bengal, India. It is also a matter of concern as continuously entering into food chain through biotic and abiotic products. The present study was conducted to evaluate the experimental effect of arsenic toxicosis on Garole sheep of West Bengal. One group was subjected to oral arsenic exposure @ 6.6 mg Kg^{-1} over 133 days when rests considered as negative control. Periodical arsenic estimation in wool, urine and feces along with hemato-biochemical alteration were checked thoroughly. It was evident from the study that long term arsenic exposure exerted a significant ($p < 0.01$) alteration compared to normal animal which were further supported by clinical abnormalities. Exposed animals showed histological changes throughout major internal organs like coagulative necrosis of liver, tubular nephritis of kidney and acanthosis of skin etc. The bio-accumulative and excretion pattern of arsenic inside body were also well understood by the arsenic estimation study of wool, urine and feces which may be helpful for discussion regarding arsenic entry into food chain via animals.

1. Introduction

Arsenic (As) is an element present ubiquitously in the earth's crust. Arsenic in drinking water (having maximum permissible limit of 0.05 μg ml^{-1}) has been recognized as a major public health concern in several regions of the world affecting not only the human population but also the livestock and agricultural products and thus entering into food chain [1]. Approximately 60 million people are at risk of arsenic exposure of Asia alone, of which 0.2 million people are exposed to arsenic endemic region in West Bengal, India. Half of the exposed people of this area exhibited the arsenic toxicosis showing skin lesion; remaining other half people are at risk due to consumption of water containing 10–12 times of minimum permissible limit of arsenic [2].

It is observed that most of the animals mainly ruminants in arsenic prone area do not show any specific clinical symptoms but from their feces and milk significant amount of arsenic is eliminated which further contaminate the pasture land and enter into human food chain [2]. Biswas et al. [3] reported that arsenic treated goats exhibited signs of toxicity from 3 week post-exposure, consisting of dullness and depression with slightly reddish coloured urine, oliguria and weakness, rough body coat with erected hairs and profound muscular weakness. Increased respiratory and heart rate were also observed after long term arsenic administration in goats. Arsenic also causes hepatotoxicity and liver damage in small ruminant like goat [4] and sheep [5]. Experimental Arsenic toxicity in sheep also affects alimentary system, adrenal system and

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respiratory system and ultimately causes death with a lethal dose of 33 mg kg^{-1} body weight in the form of Sodium Arsenite [6].

Among the small ruminant husbandry in India, Garole sheep rearing plays a crucial role in socio-economic condition of rural people of West Bengal. It carries mutated Boroola gene, characteristic of high fecundity, which implies a special economic importance to this animal [7]. The Sundarban area of South 24 Parganas and a small part of North 24 Parganas is in a deltaic zone. A previous study conducted by the School of Environmental Studies from Jadavpur University demonstrated that the extent of arsenic contamination in the ground water of South 24 Parganas was substantial. Garole sheep which live in this area are therefore chronically exposed to arsenic, and there is always a likelihood of arsenicosis in this breed which may affect consumers of affected sheep meat. Due to the paucity of information on arsenic toxicity in garole sheep, this study has been undertaken to further understanding of arsenicosis in sheep based on clinical evaluation, biochemical mining, hematological changes and histological findings. To the authors' knowledge, this is the first such study in garole sheep.

2. Materials and methods

2.1. Animals

Eight apparently healthy male Garole sheep (8–9 months of age, weighing between 10 and 13 Kg) purchased from arsenic-free villages of Kakdwip (as declared by Public Health Engineering Department, Govt. of West Bengal) were used in this experiment. They were caged individually in custom-made stainless steel metabolic cages and reared in arsenic free condition at small animal unit of Department of Pharmacology and Toxicology, West Bengal University of Animal and Fishery Sciences, Nadia, West Bengal. The animals were kept stall feeding with the supply of paddy straw and concentrates and ad lib. water.

Before starting the experiment, the animals were dewormed once with a mixture of levamisole and oxyclozanide (Fluzan, Jeps pharmaceuticals) at the dose rate of 7.5 mg kg^{-1} body weight. The animals were acclimatized in experimental environment for one month. Institution Animal Ethics Committee approved experimental protocol before starting the experiment. The animal experimentation was duly approved by the IAEC before.

2.2. Design of experiment

After conventional toning up all eight Garole sheep were randomly grouped into two groups. Four animals of group1 were kept as healthy negative control. As there is no such reference for experimental chronic dosage patterns in garole sheep so the rest four sheep (group2) were fed with sodium arsenite powder orally mixed with water @ 6.6 mg kg^{-1} b.wt. daily which is 1/5th of the lethal dose in merino sheep [5] for 133 days. Wool (from loin region), urine (by catheterization to avoid contamination) and feces (collected from metabolic cage) sample were collected every seven days interval in the early morning to find out residue of arsenic. Blood was collected aseptically by vacutainer tube from jugular vein at every 14 days interval for periodical biochemical and hematobiological analysis simultaneously.

2.3. Reagent

Diagnostic kits to assess Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum glutamic pyruvic transaminase (SGPT), Blood Urea Nitrogen (BUN) and Creatinine activity were obtained from Cogent, India. Other chemicals of analytical grade were purchased from Rankem Pvt. Ltd., E-Merck (India), and Sigma Aldrich (USA).

2.4. Estimation of total arsenic

Total arsenic was quantified by wet ashing procedure in hot plate using tri-acid mixture of nitric acid, perchloric acid and sulphuric acid (10:4:1) following the method of Dutta et al. [2]. Briefly, the digested samples were diluted with deionized Millipore water, passed through Whatman filter paper No. 4 (Rankem, India) and made the volume to 10 ml. Concentrated hydrochloric acid (5 ml) was added to it and shaken well. Then 1 ml of potassium iodide (5% w/v) and ascorbic acid (5% w/v) mixture was added and the aliquot was incubated for 45 min for transformation of arsenate to arsenite [8]. The final volume was made up to 25 ml with Millipore water and arsenic concentration read in Atomic Absorption Spectrometer (AAS) equipped with vapor generation accessories (model No. VGA77). The operating parameters were: lamp, arsenic hollow cathode lamp; wavelength, 193.7 nm; slit width, 0.5 nm; lamp current, 10.0 mA; vapor type, air/acetylene; air flow, 10.00 $Lmin^{-1}$; inert gas for hydride generation, Argon. Reducing agent (Aqueous solution of 0.6% sodium borohydride was prepared in 0.5% w/v sodium hydroxide) and 40% HCl were prepared freshly before use. The working standards were 2.5, 5, 10, 15 and 20 μgL^{-1} and prepared by same procedure as test sample.

2.5. Biochemical parameters

Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum glutamic pyruvic transaminase (SGPT) activity was measured by Reitman and Frankel [9] method using commercially available kit (Cogent, India) and following manufacturer's instructions. The activity was expressed as IU L^{-1}. BUN was measured from plasma samples by the DAM method and Plasma creatinine was estimated from plasma samples following Jaffes reaction by standard procedure depicted in manufacturer's instruction kit (Cogent, India) and both the quantities were expressed in $mgdl^{-1}$.

2.6. Hematological parameters

From blood sample hemoglobin level was determined at 14 days interval by indirect acid haematin method as described by Coffin [10] and expressed as gm/dl. Total erythrocyte count was done following standard method of Wintrobe as described by Schalm et al. [11].

2.7. Histopathology

Samples of liver, kidney, skin and intestine were collected from all the sheep in 10% buffered formal saline for histopathological examination as these organs are rich in oxidative system and more susceptible to arsenic toxicity [12].

2.8. Statistical analysis

Each of the three parameters i.e. arsenic concentration in faeces, urine and wool was analyzed by two -way ANOVA using the following linear model;

$$y_i = \mu + d_j + ex_k + dex_{j(k)l} + e_{ijklm}$$

where, y_i is the as conc. in faces/urine/wool, μ is the overall mean, d_j is effect due to days of experiment; ex_k is effect due to exposure of