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Hexavalent chromium induces testicular dysfunction in small Indian mongoose (*Herpestes javanicus*) inhabiting tanneries area of Kasur District, Pakistan

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ABSTRACT

Hexavalent chromium (Cr-VI) is widely used in tanning industries, however, being highly toxic metal, its accumulation in the animal body can adversely affect reproductive functioning. If directly discharged untreated into the environment (adjoining nullahs and soil), it can contaminate the environment. Resultantly, along with human, inhabiting wildlife species get exposed to chromium toxicity, which may enter into the animal body through drinking water and food chain. The current study investigated toxic effects of Cr (VI) on testicular functioning of adult male small Indian mongooses inhabiting the tannery area of Kasur district, Pakistan. Soil, water and animal specimens were sampled from study area on monthly basis from February 2015 to January 2016. The captured mongooses were sacrificed to collect blood and body tissues (liver, kidneys and testes) samples. The concentrations of chromium in the samples were quantified using atomic absorption spectrophotometry. Hormonal concentrations were estimated using ELISA kits. The cellular changes occurring in the testicular tissues were studied through light microscopy. Average Cr-concentrations were found significantly higher in experimental area soil and water samples, and in the blood and body tissues of the mongooses compared to control, while body and testicular weights of experimental animals were found reduced. Serum testosterone ($p = 0.037$) and luteinizing hormone ($p = 0.000$) levels were found significantly lowered while follicle stimulating hormone ($p = 0.000$) levels significantly increased in experimental animals. Histological analysis revealed disorganization of seminiferous tubules, and apparently decreased numbers of Sertoli and the Leydig cells. Spermatozoa and seminiferous tubular counts were also found significantly ($p = 0.000$) reduced compared to control. Interstitial spaces were found widened. The study concludes that Cr from tanneries environment is up taken by mongooses leading to testicular tissue damage and potential impairment of reproductive functioning of the species.

1. Introduction

The increasing concern about ecological contamination because of overwhelming use of metals has attracted active research in toxicological studies (Marques et al., 2000). Chromium, one of the most widely used heavy metals in leather tanning, chrome plating, pigment production, is present in two oxidation states (trivalent; Cr-III and Hexavalent; Cr-VI) (Japan, 2010; Kotas and Stasicka, 2000). It is widely used in many industrial processes and has prompted huge amount of polluted waste water into the environment (Wai et al., 2010). About 40% of the used chromium is directly discharged into the wastewater (Boer et al., 2004; Sharma and Goyal, 2009). Unregulated application and direct discharge of chromium in the effluents causes environmental pollution, and water and soil contamination (Szulczewski et al., 1997). Since chromium is extremely toxic metal and oncogenic, direct discharge of industrial discharges into soil and wastewater streams can lead to serious health problems to humans and wild animals (Desai et al., 2008).

Hexavalent chromium (Cr-VI) is 100 – 1000 times more toxic than trivalent elements (Stoecker, 2004). Its strong oxidizing property makes it toxic, it causes allergic dermatitis and carcinogenic effects to animals and humans exposed to this metal (Von and Liu, 1993). Li et al. (2011) investigated that chromium instigates acute and chronic toxicity including dermatotoxicity, neurotoxicity, genotoxicity, immunotoxicity, carcinogenicity along with environmental toxicity.

Hexavalent chromium is also a reproductive toxicant and can cause

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ovo-toxicity by crossing placental barrier, it may also delay puberty, extend estrous cycle and decrease follicle numbers in rodents (Kanojia et al., 1998). Aruldhas et al. (2006) and Subramanian et al. (2006) discovered azoospermia, spermatogenesis disruption, premature release of spermatocytes, phagocytosed sperms, decreased spermatozoa number and sperm motility in male monkeys (*Macaca radiata*) exposed to various chromium doses. Similarly, human and laboratory animals' exposure to chromium has shown poor semen quality, decreased sperm number, abnormal sperm morphology and reduced progressive sperm motility (Li et al., 2001) as well as increased follicle stimulating hormone (FSH), decreased testosterone and luteinizing hormone (LH) levels (Marouani et al., 2012).

Light microscopic evidence of epididymal epithelium pathology and testicular damage has already been reported (Aruldhas et al., 2004, 2005) in chromium-treated monkeys. Ernst and Bonde (1992) discovered decreased semen quality and sperm motility and adverse effects on testis and epididymal functions in rat species exposed to different chromium concentrations for various duration. Some other studies (Li et al., 2001) and (Acharya et al., 2006) quantified reduced sperm counts and elevated numbers of abnormal spermatozoa in rats and mice, respectively, while similar results were observed in rabbits (Yousef et al., 2006) and bonnet monkeys (Subramanian et al., 2006) treated/exposed to chromium.

For histo-pathological analysis, it is important to sacrifice the experimental animal and collect body tissues for any obvious alterations. Most toxicological studies these days are being conducted on vertebrate species these days, so we used the small Indian mongoose (*Herpestes javanicus*) in the current study, a small-sized wild mammal that is commonly found in the tannery area habitat (study area) in the Kassur District. Since, the population status of the species under study is 'Least Concern' (L.C), so it is ideal to utilize it in Eco-toxicological investigation, focusing on wild vertebrates, especially mammals. Being predator as well as the prey, small Indian mongoose plays important role in the ecological food chain, and also it acts as a biological control of rodents and snakes. Toxicological damage to this species can alter the structure of the food chain, in turn. That is why the small Indian mongoose was selected for the current study.

Owing to the fact that wildlife species rely on encompassing environment for food and water utilization for their survival, and also keeping in perspective the toxic nature of hexavalent chromium in the environment, the current study aimed at investigating whether male small Indian mongooses inhabiting tanneries area of district Kasur uptake Cr(VI) from the environment, and what is the sub-lethal impact of this chromium uptake on the reproductive functioning of the species exposed to Cr(VI), as a representative wildlife species in the study area. Results of the current study could be generalized to other important species occurring in similar kinds of environments.

2. Materials and methods

2.1. Study area

Tanning has a long-standing tradition in Kasur district, Punjab province of Pakistan and today it is the biggest tanning concentration in the country. District Kasur (latitude 31° 05' north, longitude 74° 31' east), comprises of approximately 3995 square kilometers (km^2) area, situated on the Lahore-Ferozepur Road at a distance of 55 km from the main Lahore city (Fig. 1). Geographically, the study area has Lahore district on the north side, Sheikhupura district on north-west and Okara district on south-west while on south-east it has Indian Territory. The study District comprises of three Tehsils including Kasur, Pattoki and Chunian. The climate is comparatively cold in winter but hot in summer. In the months of May and June, the temperature may rise up to 44 °C. Underground water is brackish due to water logging and salinity. An area of 13,782 acres is under forests (Pakistan, 2012).

In Kasur district, the tannery industry is most important, where there are more than 364 active individual tanning industrial units (Pakistan, 2012). A tannery is the place where the skins and hides of animals are processed to produce leather through leather tanning process, which permanently alters the protein structure of the skin, making it more durable and less susceptible to decomposition, and also possibly coloring it (Punjab, 2002). As a result of tanning activities, about 150 t′ solid waste and about 9000 m^3 of contaminated waste water are being discharged on daily basis to main streams, which has resulted in pollution of the natural environment of the area leading to serious health hazards to native communities (human being and animals) directly exposed to contaminated ground water, crops and unhygienic environmental condition (Pakistan, 2012).

2.2. Sample sites

Potential habitat of the small Indian mongoose was explored in the study area through reconnaissance survey; five sampling sites were selected (Fig. 1) and visited on monthly basis for data collection from February 2015 to January 2016. The sampling Site-I (Niaz Nagar) included the main industrial area where the leather tanning was being processed, site-II was the outlet of the industrial area Niaz Nagar, site-III included Pind Qaisar Garh near Kasur Tanneries Waste Management Agency (KTWMA inlet), and site-IV was KTWMA outlet, while Site-V was Iqbal Nagar which included Dada industries, a small industrial estate (Fig. 1).

All five experimental sampling sites (in the study area) were not very far away from one another and so were equally toxic, having same habitat features. Moreover, the main focus of the current study was to investigate the toxic effect of chromium from the tannery area environment onto the reproductive status of the small Indian mongoose, exposed to chromium contaminated habitat.

2.3. Sample collection

Samples of nullah water, soil (sludge) and blood and body tissues of mongoose were collected from February 2015 to January 2016. Each month five water samples were collected in sterilized air tight plastic bottles, properly labeled and stored in ice-box to avoid contamination and degradation and brought into the laboratory for analysis. Similarly, five soil samples were taken each month from the sampling sites by digging a six inches deep V-shaped hole using a shovel. Collected soil samples were immediately transferred to sample bags and zip locked to avoid contamination.

For collection of body tissues of the representative wildlife species in the study area, three live mongooses (adult male) were captured every month, using especially designed mesh traps at selected sampling sites and brought to the laboratory. We, for current analysis captured all adult mongooses from the field, and the age was assessed by their body size, body weight and external body measurements (head-tail length, tail length, body length between head and tail and snout length), the immature and sub-adult animals trapped were not included for the study and analysis and so they were released back to their environment. The captured animals were euthanized with chloroform and after recording their external body measurements, the specimens were sacrificed. The euthanized animal was placed on its supine position in the wax filled dissection tray, a ventral midline incision was made from the pubic region to the mandibular symphysis, the skin was reflected to the level of the backbone on right and left sides, it opened the abdominal cavity along the ventral midline to expose the internal organs and to collect blood and body tissues including testes, liver and kidneys. Blood was directly drawn by cardiac puncture and centrifuged (3500 rpm for 15 min) to obtain plasma while body tissues of the male mongooses were processed for atomic absorption spectrophotometry and histological procedure.

The control samples for the current study were collected from the Potohar Plateau (Fig. 1), an area not known for having any tannery