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Embryotoxic and Teratogenic Effects of Hexavalent Chromium in Developing Chicks of *Gallus domesticus*

S. N. Q. Asmatullah, A. R. Shakoori

Department of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore 54590, Pakistan

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Heavy metals as components of industrial effluents such as mineral pigments, scouring effluents, sludge and tannery effluents cause health hazards, including embryotoxic, teratogenic and carcinogenic effects in a large biota (Ferm 1972; Nriagu and Pacyna 1988). Reduction in embryonic growth in externally exposed eggs of mallard (Hoffman and Eastin 1981), embryo lethality and developmental abnormalities in the Japanese medaka (Copper and McGeorge 1991), and gross developmental malformations in chick embryos such as reduced body size, micromelia, twisted neck, everted viscera, hemorrhage and microphthalmia (Gilani and Alibhai 1990) have been attributed to heavy metals. Chromium is one of the major components of effluents from tanneries, which is posing serious threat to human and animal health in Kasur, an industrial town in suburbs of Lahore, Pakistan. Higher concentrations of chromium are known to be toxic, carcinogenic, and mutagenic (Petrilli and Deflora 1977; Cary 1982; Nair and Krishnamurthi 1991). Chromium has also been reported to cause teratogenic and embryotoxic effects such as resorptions of embryos, external abnormalities, cleft palate and hydrocephaly in hamsters (Gale 1982).

The trivalent and hexavalent states of Cr differ markedly in a number of their biological properties (Levis and Bianchi 1982). The hexavalent form (Cr VI) is more toxic and mutagenic than the trivalent form (Cr III). Derivatives of Cr III are water soluble at neutral pH and can be removed from medium in the form of chromium hydroxide, while Cr VI forms are highly insoluble (Levis and Bianchi 1982; Ohtake et al. 1990; Vishnyakov et al. 1992; Yamamoto et al. 1993). Chromium VI had been found teratogenic in frog tadpoles which led to abnormal behavioral responses and death (Abbasi and Soni 1984).

In the present investigation the embryotoxic and teratogenic effects of hexavalent chromium was studied in the developing chick embryo.

MATERIALS AND METHODS

One hundred fertilized eggs of white leghorn breed of *Gallus domesticus* were purchased from the Veterinary Research Institute, Lahore, Pakistan and divided into 5 groups, each of 20 eggs. Three groups were administered with 0.1 ml/egg of different concentrations of aqueous solutions of $K_2Cr_2O_7$ containing 25, 50, and 100 µg chromium. The other two groups which were vehicle control and control were respectively administered with 0.1 ml of distilled water and no treatment. For chromium administration the eggs were randomly selected and cleaned with a piece of cotton soaked in 70% alcohol. A small window, for insertion of needle, was made in the shell of each egg, except control eggs,

Correspondence to: A. R. Shakoori

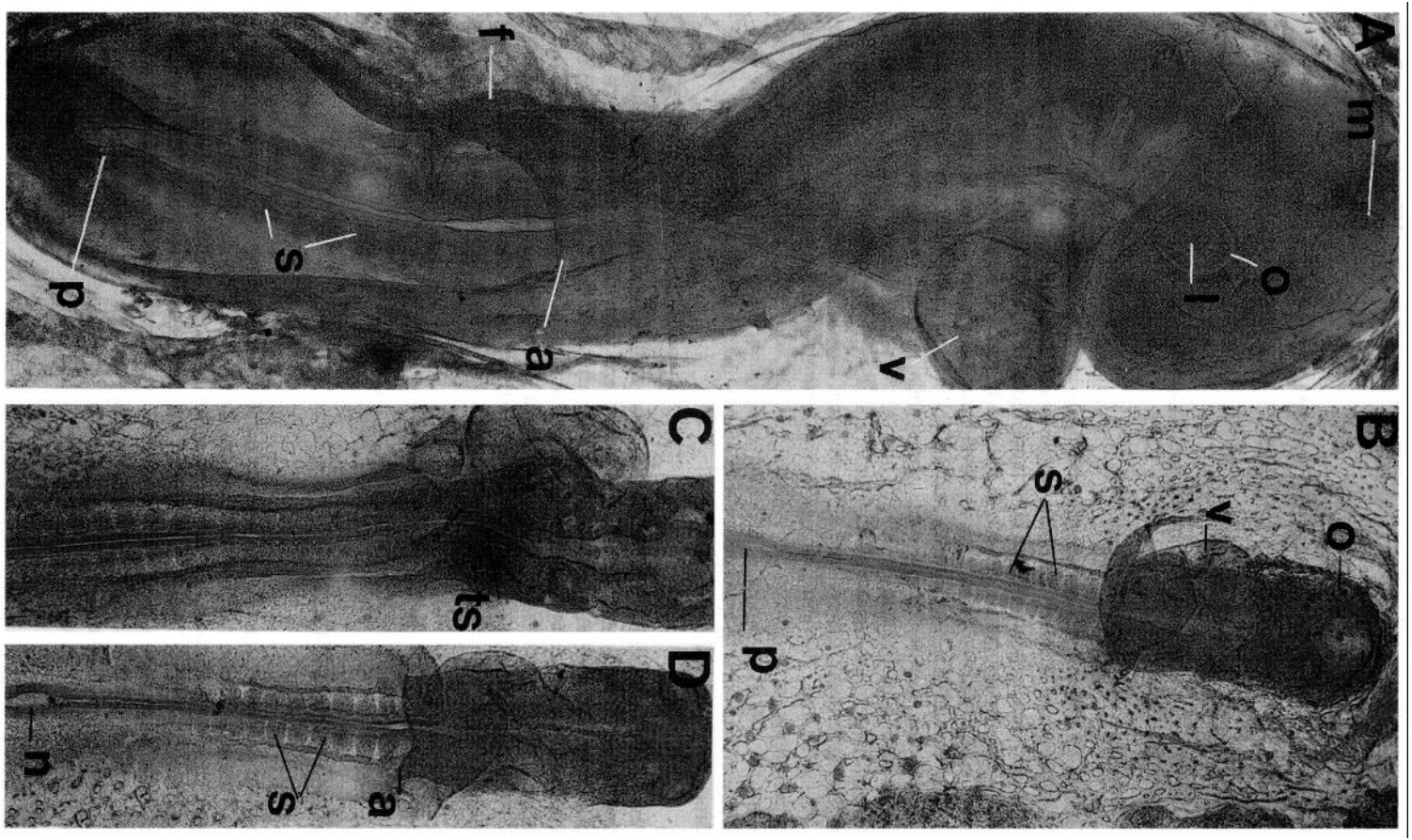

Figure 1: Three days old chick embryos. A, a member of the control group showing normal development; B, C, D, embryos extracted from eggs treated with 25, 50 and 100 µg $K_2Cr_2O_7$, respectively indicating reduction in body size and abnormal development of other body parts. a, amnion; f, forelimb; 1, eye lens; m, mesencephalon; n, patent neural tube; o, optic cup; p, primitive streak; s, somite; ts, twisted spinal cord; v, heart ventricle. Magnification: 40X; Staining: Borax carmine.

without rupturing shell membrane. Using a micro-applicator, 0.1 ml of each concentration was injected into the yolk of each egg under sterile conditions. Following injection, the hole in the egg shell was sealed with liquid paraffin wax. After treatment the eggs were placed in an incubator adjusted at 38+0.5°C. Humidity was provided by placing a water filled beaker in each shelf of the incubator. The eggs were rotated twice a day.

The embryos were recovered on 3rd day of incubation, fixed in freshly prepared Bouin's fixative and the whole mounts prepared by routine borax carmine staining procedure. For histological studies embryos were recovered on 7th day of incubation, fixed in Bouin's fluid and embedded in paraffin wax. Sections (6-8 µm thick) were stained in haematoxylin and eosin. Prepared slides were studied under stereomicroscope for changes in body size, which was measured directly from the photographs taken at the same magnification with the help of a scale, and any malformations in curvature of brain parts, optic cup, eye lens, otic vesicle, somites, heart, neural tube, spinal cord and haemopoiesis. Selected embryos were microphotographed with the help of camera fitted Olympus stereomicroscope. For each observation 20 slides of embryos were studied.

RESULTS AND DISCUSSION

The prepared whole mount slides of chick embryos, recovered at day 3 of incubation, were studied for embryotoxic effects of chromium. The control embryonic brain was distinguishable into prosencephalon, mesencephalon and rhombencephalon (Fig.1A). The curvature of brain parts was according to the stage 18 explained by Matthews (1986). The eye cup with lens vesicle was prominent. Otic vesicle was also developed. The cardiac development with a very prominent ventricle was still in the neck region. The somites were quite distinguishable and posterior region was still not covered by amnion. The neural tube was almost complete with a small opening at posterior of the embryo showing regression of primitive streak (Fig.1A). The development of embryos treated with only distilled water was similar to controls.

The eggs treated with different concentrations of Cr VI showed abnormal development. In the case of 25µg/egg dose group, the brain was not well developed with unfolded brain parts (Fig.1B). Only optic vesicle was formed from diencephalon. The heart primodia protruded from neck region as a tubular structure. The somites development was abnormal. The neural tube was twisted and was not closed at anterior region (Fig.1B). In higher dose groups (50 and 100 µg/egg), the embryos were more negatively affected with reduced body size, abnormal development of brain parts, somites, heart, haemopoiesis and neural tube (Fig.1 C, D).

In control embryos the eye components viz. eye lens, retina and optic nerve were well developed (Fig.2 A), while in treated groups the eye was defective with undifferentiated cornea, eye lens and retina (Fig.3 B-C). The spinal cord was quite normal in control chicks (Fig.2 D). At 100 µg/egg, the gray matter and white matter were not properly differentiated (Fig.2 F), whereas in lower dose (25 µg/egg) the spinal cord was differentiated, though twisted with abnormal elongation of one horn (Fig.2 E). The heart was quite well differentiated in control chicks (Fig.3 A). It had well developed atria and ventricles with complete interventricular septum. The ventricular walls had well developed myocardial layer. When these heart structures were studied in treated chick embryo, heart tissue was found to be affected adversely in all groups. For example, development of tubular heart, thinning of myocardium, stenosis of atrio-ventricular valve

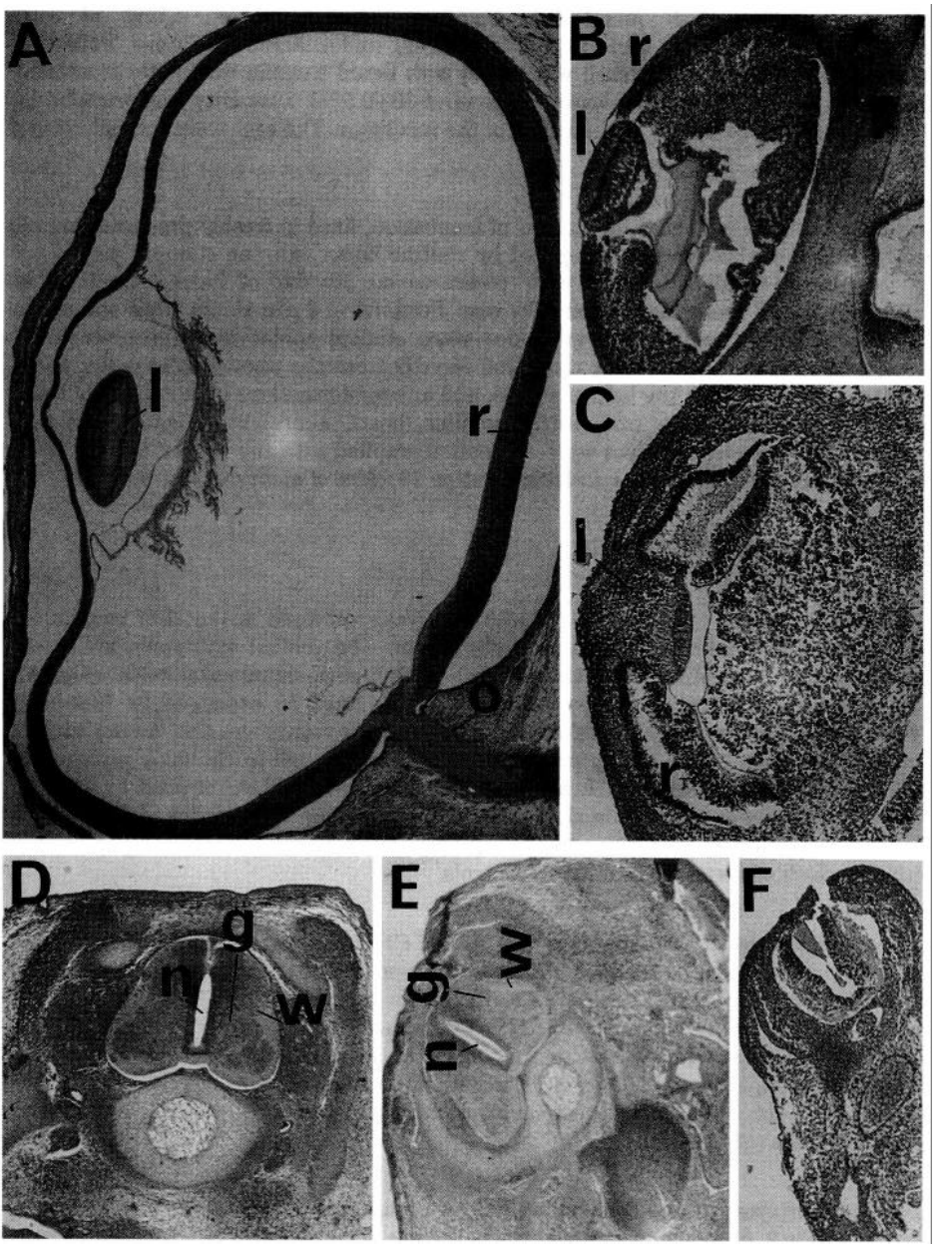

Figure 2. Effect of hexavalent Cr on the development of chicken eye; A, control; B, 25 µg $K_2Cr_2O_7$ administration; C, 100 µg $K_2Cr_2O_7$, and spinal cord; D, control; E, 50µg $K_2Cr_2O_7$ administration; F, 100 µg $K^2Cr_2O_7$; g, gray matter; 1, eye lens; n, neurocoel; o, optic nerve; r, retina; w, white matter. Magnification: 40X, Staining: H and E.

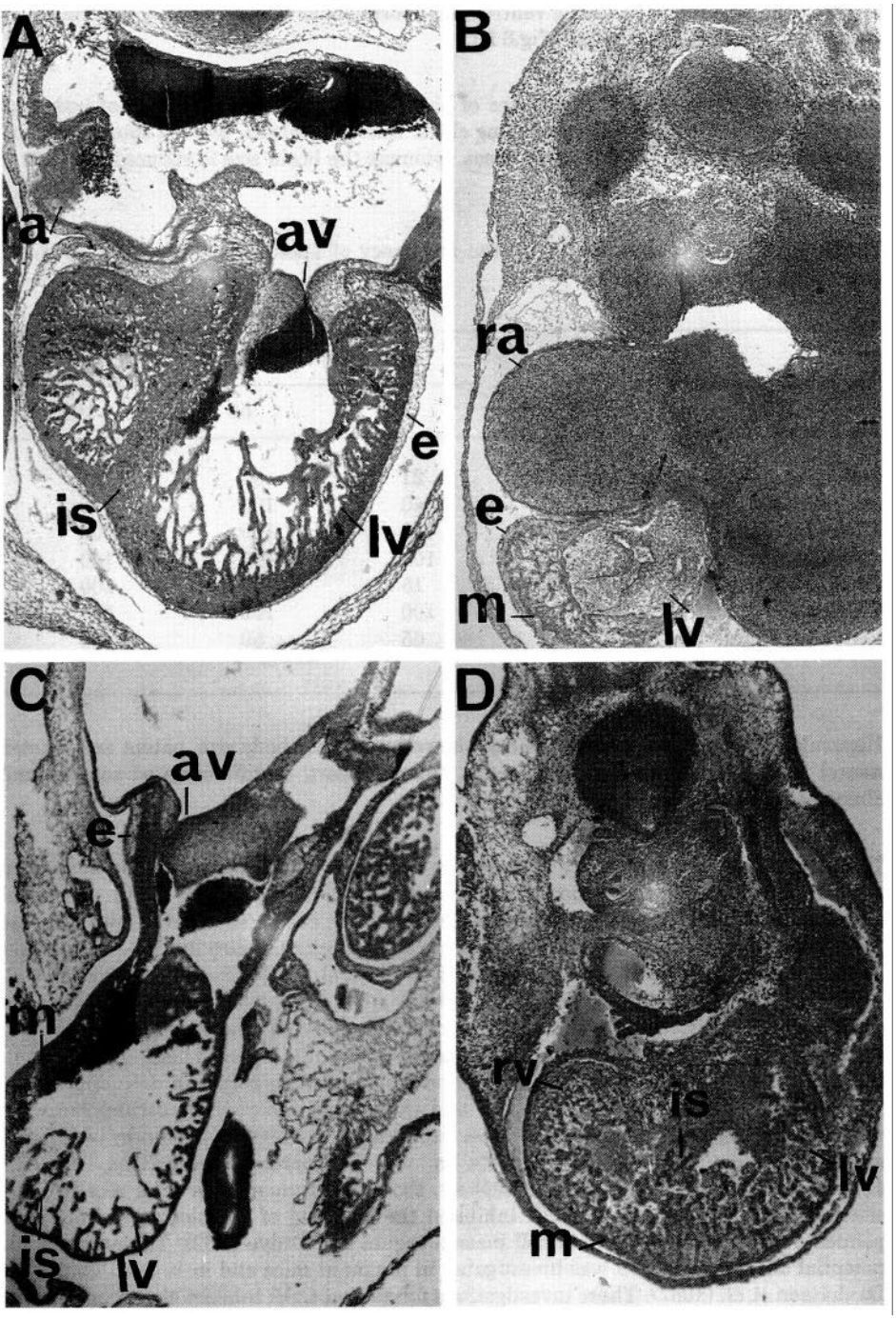

Figure 3. Effect of hexavalent Cr on the developing heart of *Gallus domesticus;* A, control; B, 25 µg $K_2Cr_2O_7$; C, 50 µg $K_2Cr_2O_7$; D, 100 µg $K_2Cr_2O_7$. av, Atrio-ventricular valve; e, epicardium; is, interventricular septum; la, left atrium; lv, left ventricle; m, myocardium; ra, right atrium; rv, right ventricle. Magnification: 40X, Staining: H and E.

(Fig.3 C), fibrous mass in place of ventricles, abnormally developed aorta, and atria (Fig.3 B), and ventricular septal defect (Fig.3 D).

Table I shows dose related frequencies of abnormalities such as reduction of body size and patent neural tube in the developing chick. Microphthalmia, twisted spinal cord and cardiac anomaly occurs 100 % at all doses, whereas the brain size is reduced 60-65 % at all doses.

Table I. Effect of hexavalent Cr on the frequency of anomalies observed at different doses in the developing chick.

Embryonic anomalies	$K_2Cr_2O_7$ administration (µg/egg)		
	25	50	100
Reduction in crown rump length (%)	21	34	47
Microcephaly (%)	40	100	100
Microphthalmia (%)	100	100	100
Twisted spinal cord (%)	100	100	100
Patent neural tube (%)	15	60	100
Cardiac anomaly (%)	100	100	100
Reduction in brain size (%)	65	59	63

Hexavalent chromium is embryotoxic as shown by reduced body size, patent and twisted neural tube, undifferentiated brain parts, tubular heart, undifferentiated somites and abnormal haemopoiesis in all Cr treated groups.

These results are in conformity with earlier reports in which heavy metals have been reported to induce teratogenecity in chicks (Gilani and Alibhai 1990). Maternal administration of Cr through drinking water increased embryonic deaths with increase in concentration and caused complete absence of implantation sites in mice. The fetuses had external and skeletal malformations (Trivedi et al. 1989). Recently, Junaid et al. (1995, 1996) found CrVI to be embryo- and fetotoxic in mice. Different concentrations of CrVI (250, 500 and 750 ppm as potassium dichromate) were administered via drinking water during days 6-14 of gestation, Reduced fetal weight, retarded fetal development, high incidence of dead fetuses and resorptions, significant increases in drooping wrists, subdermal haemarrhagic patches, kinky and short tails, and reduced ossification were found in highest dose groups. In another study by Gale (1982), chromium was found embryotoxic in hamster. The anomalies were embryonic resorptions, external abnormalities, cleft palate and hydrocephaly. Blastocyst formation in mice was strongly affected by CrVI compounds which inhibited the hatching of the blastocyst from zona pellucida and formation of inner cell mass (Jacquet and Draye 1982). The embryotoxic potential of CrVI and CrIII was investigated in pregnant mice and in *in vitro* studies by Danielsson et al. (1982). These investigations found that CrVI inhibits chondrogenesis at concentrations of 0.1 µg/ml medium, whereas CrIII did not show any overt cytotoxicity, although it accumulated in the fetus in high enough concentrations to have a direct effect on the embryonic structures.

Treatment of Chinese hamster ovary cells with 150 and 300 µM Na_2CrO_4 for 2 hr decreased colony forming efficiency by 46 and 92%, respectively. These treatments induced dose-dependent intranucleosomal fragmentation of cellular DNA which caused cell death. It was suggested that the persistant Cr-DNA adducts may be responsible for the cell death due to cell cycle delay and transcriptional inhibition caused by chromium (Manning et al. 1994).

Chromium (VI) compounds are potential toxic and carcinogenic metals (DeFlora et al. 1990). With respect to toxicity, hepatic and renal toxicity have been reported either in workers or in animals exposed to CrVI (Goyer 1990; Hojo and Satomi 1991). Chromium (VI) compounds also induced DNA damage *in vivo* (Coogan et al. 1991) and in cultured cells (Sugiyama et al. 1986) as well as selectively inhibiting the activity of enzymes such as glutathion reductase in mammalian cells (Sugiyama et al. 1989) by forming paramagnetic chromium such as CrV and CrIV (Sugiyama 1994; Kawanishi and Hiraku 1995; Sudgen and Wetterhahn 1996). The present study highlights teratogenic effects on the developing chicks exposed to even very low concentrations of $K_2Cr_2O_7$.

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