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Therapeutic Effect of Guava Fruit Extract on Cadmium Induced Toxicity in Developing *Mus musculus*

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ABSTRACT

During this study, guava fruit extract was tested for its ameliorative effects on congenital defects induced by cadmium in developing mice. For this purpose, different aqueous concentrations of cadmium chloride *i.e.* 0.00, 6.25, 12.50, 25.00 µg/g B.W of mice were given orally to pregnant mice on days 6-12 of gestation daily. Another series of mice were treated with different concentrations of cadmium chloride and also with an antidote (guava fruit extract) on days 6-18 of gestation daily once a day. Fetuses were recovered on day 18 of gestation, analyzed based on morphological, morphometric, histological and skeletal characteristics. Morphometric analysis of the cadmium treated groups indicated a significant ($p<0.05$ to $p<0.001$) reduction in fetal body weight, crown rump length, head circumference, eye circumference, forelimb and hind limb lengths and tail size compared to control fetuses. Morphological studies of fetuses showed abnormalities like micromelia, skeletal defects, hemorrhages, displacement of limbs, open eyelids and distorted axis. Histological defects included dilation and absence of ventricles, underdeveloped cerebellum, microcardia, necrosis in atrium, underdeveloped ventricles, hydropericardium and necrosis of the liver. Skeleton analysis of cadmium treated groups showed mostly un-ossified skeletons. The control mice showed normal ossification as compared to experimental groups. Morphological, histological and skeletal studies of fetuses treated cadmium chloride along with guava fruit extract revealed ameliorative effects on various kinds of birth defects. It is concluded based on this current study that guava fruit extract exerted a therapeutic effect on developmental anomalies induced by cadmium exposure in prenatal mice.

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Asmatullah and CA planned the study and wrote the manuscript. MT and SA performed experimental work. BN did photography. MA helped in histology.

Key words
Cadmium, Developmental defects, Guava extract, Therapeutic effect, Developing mice.

INTRODUCTION

Cadmium a heavy metal and an important pollutant that is present in everywhere in the environment including water, air, soil and food. About ten times less cadmium is added to the environment by natural sources compared to anthropogenic sources (Irwin *et al*., 2003). Food is the major source of human exposure to cadmium, as plants can absorb cadmium from the soil .Subsequently, cadmium can easily incorporate into different plant originated foodstuff (Petersson *et al*., 2004).

Cadmium potentially can cause serious environmental hazards including embryotoxicity, carcinogenicity and teratogenicity in developing embryos, which may lead to the serious abnormalities such as reduction in body weight, micromelia, micropthelmia and hemorrhages (Gilani and Alibhai, 1990). Cadmium plays its role as a teratogen by transforming the placenta structurally and functionally (Wang *et al*., 2012).

Absorption and function of cadmium in the human body largely depends upon the divalent and trivalent species of metal and mineral deficiencies such as iron and calcium in the body during pregnancy (Waalkes, 2000). Lower doses of cadmium cause gastrointestinal dysfunctions and diarrhea. Higher doses of cadmium affect the nervous and cardiovascular systems and accumulate in liver and kidney, which causes renal tubular dysfunction and leads to renal failure. The cadmium in the kidney alters vitamin D metabolism, which may disturb the calcium balance and leads to bone diseases (Hiratsuba *et al*., 1999).

The damage caused by teratogens minimized by the administration of other chemical agents, which counteracts and neutralizes the action or effect of toxic substances these agents known as antidotes (Robert, 1998). Selenium (Se) is an essential metal that has antioxidant properties and it can be useful against cadmium toxicity (El-Boshy *et al*., 2015). Vitamin C also plays an important role in reducing cadmium induced toxic effects. Guava fruit is one of the fruit having vitamin C that has been used in this study.

Guava fruit contains 13-26% dry matter, 0.5-1 % ash, 74-87% moisture, 0.4-0.7% fat and 0.8-1.5% protein (Chin and Yong, 1980). Guava is also a major source of antioxidants that help in fighting against degenerative

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diseases such as heart diseases, inflammation, cancer and inappropriate functioning of the brain (Koo and Mohamed, 2001). Major antioxidants present in guava are carotenoids and polyphenols. Nearly 2.62-7.79% of extractable polyphenols are present in guava fruit (Matsuo *et al*., 1994). Guava is also a good source of ascorbic acid (vitamin C). A single guava fruit contains four times more vitamin C as compared to an orange (Hui, 2006). In immune cells, a higher concentration of vitamin C is present i.e. utilized by immune cells quickly during any type of infections. They also have the scavenging ability to reduce the free radicals and maintain the chelating reactions of metals (Preedy *et al*., 2010).

The above description showed that antioxidants and ascorbic acid (vitamin C) play a vital role in reducing degenerative diseases and the free radicals to maintain the chelating reactions. So, in order to examine whether guava fruit extract which contains many antioxidants, vitamin C, has the ability to reduce the toxic and teratogenic effect produced by the heavy metal cadmium, the effect of cadmium on mice and the ameliorative activity of guava fruit extract against cadmium toxicity was examined in the following experiment.

MATERIALS AND METHODS

Sexually mature Swiss Webster albino mice *Mus musculus* of age 8-9 weeks were used during this study. The animals were kept under standard conditions, maintained according to the standard protocols of the approved animal treatment conditions of the medical ethics committee of Punjab University, Lahore, Pakistan. Standard conditions included 12 h dark and light cycle, room temperature 27±2°C. Animals were housed in 12”x16” steel cages and provided with the National Chick Feed# 12 and water *ad libitum*.

Females in estrous were caged with males of the same breed. The females were carefully examined next morning for the presence of vaginal plug. That day was taken as day 0 of gestation. A total of 40 pregnant females were grouped as control, vehicle control, cadmium treated and cadmium with antidote groups.

Cadmium chloride was purchased from (BDH Analar Chemical Industry). The doses were prepared by dissolving the salt in distilled water in such a way that 0.1 ml of solution contained desired concentration of the chemical. Antidote was prepared by blending the guava fruit (500g+300 ml distilled water). This paste was then centrifuged to remove fibers and other particles and the supernatant was used further for experimentation.

Three different dose concentrations of cadmium chloride, 6.25, 12.50 and 25.00 µg/g B.W., were given to pregnant mice. These doses were given with the help of 1 ml plastic syringe attached with a rubber tube, which was specially prepared for oral feeding. These doses were given at days 6-12 of gestation daily, once a day, in addition to guava fruit extract. The guava extract was continually given up to day 18 of gestation.

On day 18 of gestation, the treated females were weighed and anesthetized with anesthetic Ether (Urban and Bleckwenn, 2002), then they were sacrificed under prescribed ethical procedures. Gravid uteri were exposed by giving midline incision. The numbers of implantation sites were recorded. The fetuses were removed from uteri and weighed. Then these fetuses were fixed in Bouin’s fixative for 48 h at 27±2°C for histological and 95% ethanol for skeletal preparations.

The morphological studies were done to record anomalies of craniofacial, trunk, limbs and tail region. The selected abnormal fetuses were photographed by using dissecting microscope Labomed, CZM6, of Japan and camera, Panasonic TZ15 (Arshad *et al*., 2017).

The morphometric studies involved recording of fetal weight, crown rump length, head and eye circumferences, length of fore and hind limbs and tail length. All the measurements were made by analytical balance and digital vernier caliper. The head and eye circumference values (p = mm²) were calculated for each fetus with the help of a computer based program the Ellipse Circumference Calculator (http://www.csgnetwork.com/glossaryc.html#calculator). The morphometric data were subjected to ANOVA by using SPSS software version 16. For the multiple comparison in treatment groups post hoc Tukey test was applied.

The analysis was done on 95% confidence interval and at minimum levels of significance $p < 0.05$.

Table I.- Embryotoxic effects of cadmium chloride on the 18 day old mouse fetuses recovered from pregnant mice exposed to different concentrations (6.25, 12.5 and 25.0 µg/g B.W) on days 6 to 12 of gestation.

Dose (µg/g B.W)	No of fetuses recovered (n)	Malformed fetuses (%)	Resorbed fetuses %)
0.00 (C)	50	0.00	0.00
6.25	32	60.85	19.23
12.5	28	68.48	26.09
25.0	20	71.85	26.63

For histological observations selected fetuses from different groups were processed for routine paraffin sectioning. The sections were stained with hematoxylin and eosin (Spencer and Bancroft, 2008) and selected sections were photographed with microscope Labomed, CZM6, of Japan and camera, Panasonic TZ15.

For skeletal preparations, the fetuses were preserved in 95% ethanol; they were eviscerated through a small abdominal incision. These eviscerated fetuses were placed

in 2% KOH solution till removal of flesh and visibility of bones. KOH used only to remove the flesh. Alizarin Red S stains was used for 30 min (using a 1% aqueous solution made alkaline by adding 2 to 3 drops of 1% KOH). The deeply stained fetuses were placed in 1% KOH until the skeleton was clearly visible through surrounding tissue and extra stain was removed. These stained skeletons were further cleared in 20% glycerinand 1% KOH (Kawamura *et al*., 1990). The skeletons were preserved in 50% ethanolic glycerol for microscopic observations and selected specimens were photographed.

RESULTS

During the present study, a significant ($p < 0.001$) increase in percentage of malformed and resorbed fetuses observed in all cadmium treated groups as compared to control. On the other hand, in antidote treated groups, number of malformed and resorbed fetuses with increasing dose concentration detected, but these are not significantly different from control or vehicle control group (Tables I, II).

Table II.- Embryotoxic effects of antidote on 18 day old mouse fetuses recovered from pregnant mice exposed to different doses of cadmium + guava fruit extract on day 6 to 12 of gestation.

Dose$^{\Delta}$ (µg/g B.W)	No. of fetuses recovered	Malformed fetuses (%)	Resorbed fetuses %)
0.00 (VC)	49	0.00	0.00
6.25	46	6.25	0.00
12.5	43	16.27	0.00
25.0	40	22.50	7.50

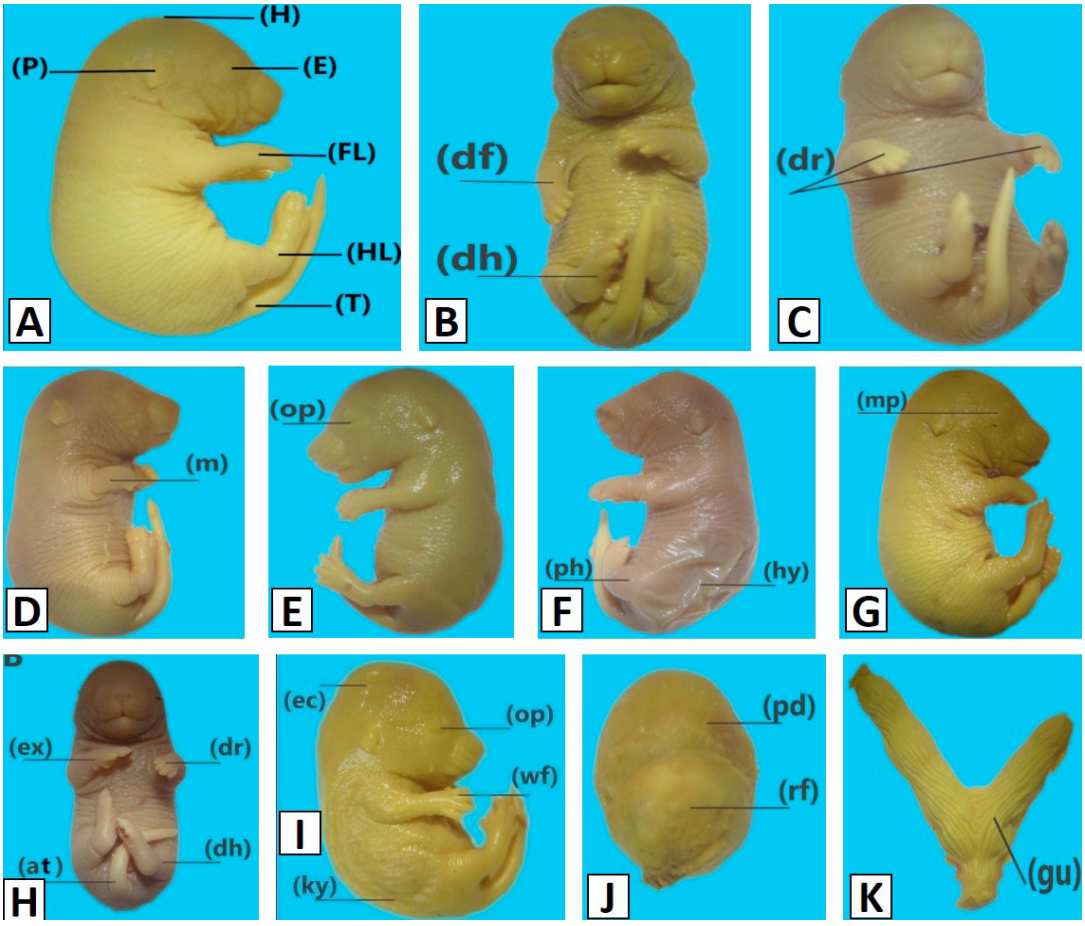

Fig. 1. Effect of Cadmium Chloride on the development of *Mus musculus;* **A,** control; **B, C, D and F,** 6.25 µg/g B.W; **E, F, G and H,** from 12.5 µg/g BW; **I, J and K,** from 25.0 µg/g BW. H, head; E, eye; FL, fore limb; HL, hind limb; P, pinna; T, tail; df, displaced fore limbs; dh, displaced hind limbs; dr, drooping wrist; m, micromelia; op, open eyelid; hy, hygroma; ph, phocomelia; mp, macro ophthalmia; ex, extension of limb; ec, exencephaly; wf, webbed fingure; ky, kyphosis; at, angular tail; pd, placental disc; rf, resorbed fetus; gu, gravid uterus. Magnification, A-H (7X), I-K (12X); Age of embryos, 18 days.
No stain used rather embryos were fixed in Bouin's solution that is why yellowish colour

Table III.- Developmental anomalies induced by cadmium in 18 day old mouse fetuses recovered from pregnant mice, administrated orally with different concentrations on days 6 to 12 of gestation.

Dose[A] (µg/g B.W)	Axis (%)	Brain (%)	Eyes (%)	Forlimbs (%)	Hind limbs (%)	Skin (%)	Skeleton defects (%)	Tail (%)
0.00 (C)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(D-I)6.25	Distorted axis (10.01)	0.00	Open eyelids (14.00)	Limb displacement (23.10) Micromelia (13.61) Drooping wrist (12.08) Extension (11.29)	Limb displacement (13.20) Micromelia (14.08)	0.00	0.00	0.00
(D-II)12.5	Distorted axis (25.20)	Hydrocephaly (9.43) Exencephaly (8.06)	Open eyelids (43.30) Microphthalmia (14.00)	Limb displacement (34.10) Micromelia (14.21) Drooping wrist (10.0) Extension (9.72)	Limb displacement (25.00) Phocomelia (13.21)	Hygroma (45.20)	Kyphosis (8.32)	Angular tail (23.32)
(D-III)25.0	Distorted axis (27.30)	Exencephaly (10.90)	Open eyelids (47.23)	Limb displacement (40.10) Extension (14.21) Drooping wrist (9.01)	Limb displacement (32.00) Webbed feet (10.11)	Subcutaneous hemorrhage (57.70)	Kyphosis (10.24)	Angular tail (33.34)
(A.D)+6.25	Distorted axis (12.54)	0.00	Open eyelid (10.47)	Drooping wrist (5.71)	0.00	0.00	0.00	0.00
(A.D)+12.5	0.00	0.00	Open eyelid (11.78)	Drooping wrist (12.20)	0.00	14.72	0.00	5.45
(A.D)+25.0	Distorted axis (15.54)	0.00	Open eyelid (17.05)	Micromelia (9.50)	0.00	Hemorrhage (10.88)	0.00	0.00

Table IV.- Morphometric analysis of 18 day old mouse fetuses (Mean±SEM) recovered from pregnant mice, administered orally with different concentrations of cadmium (6.25, 12.5 and 25.0 µg/g B.W) and antidote +cadmium on days 6-12 of gestation.

Dose (µg/g B.W)	Body weight (mg)	C.R length (mm)	Head circumference (mm^2)	Eye circumference (mm^2)	Fore limb length (mm)	Hind limb length (mm)	Tail length (mm±S.E)
C	1277.24±74.25	27.95±2.22	23.48±1.30	3.97±0.45	6.67±0.50	7.29±0.80	7.33±1.26
V.C	1272.52±100.12	26.19±2.05	20.78±0.61	3.90±0.57	6.60±0.60	7.45±0.58	7.42±0.61
6.25	1016.40±290.58**	19.20±3.03**	12.47±0.87***	2.80±0.29***	6.28±1.10**	6.12±1.45*	6.59±0.84**
A.D + 6.25	1235.80±128.50	20.01±2.88	14.01±1.12	3.80±0.51	6.76±1.32	7.32±1.65	7.09±1.42
12.5	876.4±183.31**	17.51±2.25**	10.97±0.194***	2.41±0.38***	5.41±0.54**	6.05±0.74*	5.44±0.59**
A.D + 12.5	1042.60±187.83	18.49±3.49	11.88±0.63	3.41±0.41	5.81±0.45	7.00±0.96	6.98±1.12
25.0	812.8±119.82**	14.85±2.48**	9.08±1.97***	1.98±0.54***	5.29±2.16**	5.59±1.23*	4.16±0.89*
A.D + 25.0	886.20±125.96	17.19±3.15	10.80±0.76	3.18±0.47	5.45±2.12	6.85±1.46	6.69±1.22

Data are expressed as parameter size ±standard error. Asterisks show significant difference against controls, ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.
C, Control; V.C, vehical control; A.D, antidose.

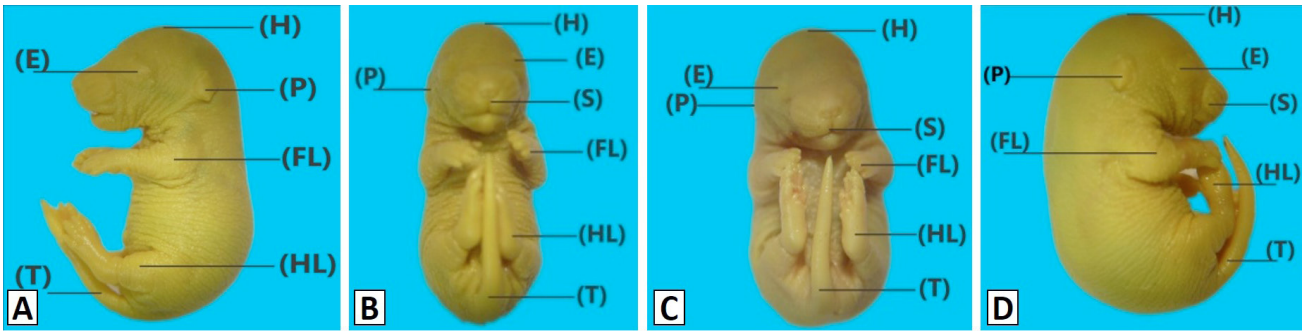

Fig. 2. Macrophotographs of 18-day-old mouse fetuses recovered from mothers exposed to different doses of Cadmium on days 6 to 12 of gestation. **A,** from vehicle control; **B,** from antidote +6.25 µg/g BW; **C,** from antidote + 12.5 µg/g BW; **D,** from antidote+ 25.0µg/g BW. H, well developed head; E, well-formed eyes with closed eyelids; FL, well developed forelimbs; P, well developed pina; HL, well developed hindlimbs; T, well developed tail; op, open eyelids; dh, distorted hindlimbs; a, acheiria, m, meromelia, ec, exencephaly; gu, gravid uterus with resorbed fetuses; wt, wrinkled tail; m, micromelia, ph, phocomelia, ky, kyphosis; at, angular tail; hy, hygroma; rf, resorbed fetus; oe,openeyelid; dr, drooping wrist; wf, webbed feet; ec, excencephaly; pd, placental disc, ex, extended forlimb; df, distorted forelimbs; mp, microphthalmia.

The morphometric observations during this study showed a significant ($p < 0.01$) reduction in mean body weight of the fetuses, CR, forelimb and tail lengths was observed in all dose groups 6.25, 12.5 and 25.0 µg/g B.W. In eye and head circumference a significant difference ($p < 0.001$) was present and hind limb length was reduced significantly ($p<0.05$) in all dose groups as compare to control group. Whereas in the antidote treated groups, the morphometric parameters were not significantly different as compared to vehicle control (Table IV).

Morphological analysis showed that the fetuses recovered from the control group had normal sized and well developed body organs (Fig. 1A). In vehicle control fetuses were also well developed, similar to control fetuses (Fig. 2A). The fetuses from dose group 6.25 µg/g B.W showed morphological anomalies like distorted forelimb (23.10%) (Fig. 1B), distorted hind limb (13.20%) (Fig. 1B), drooping wrist (12.08%) (Fig. 1C), forelimb micromelia (13.61%) (Fig. 1D), open eyelids (14.00%) (Fig. 1E), hindlimb micromelia (14.08%), distorted axis (10.01%) and extension of forelimb (11.29%). The fetuses from dose group 12.50 µg/g B.W showed morphological anomalies like phocomelia (13.21%) (Fig. 1F), hygroma (45.20%) (Fig. 1F), microphthalmia (14.00%) (Fig. 1G), drooping wrist (10.0%) (Fig. 1H), angular tail (23.32%) (Fig. 1H), extension of forelimb (9.72%) (Fig. 1H), distorted hindlimb (25.00%) (Fig. 1H), distorted axis (25.20%), hydrocephaly (9.43%), exencephaly (8.06%), kyphosis (8.32%) and hygroma (45.20%). By studying under binocular dissecting microscope and by histological examination resorbed embryos are confirmed.

The fetuses from dose group 12.50 µg/g B.W showed morphological anomalies like excencephaly (10.90%) (Fig. 1I), open eyelids (47.23%) (Fig. 1I), webbed foot (10.11%) (Fig. 1I), kyphosis (10.24%) (Fig. 1I), distorted axis (27.30%), distorted forelimb (40.10), distorted hindlimb (32.00%), extension of forelimb (14.21%), drooping wrist (9.01%), hemorrhages (57.70%) and angular tail (33.34%), Fetuses recovered from cadmium chloride+antidote group showed normal growth of eyes, brain, forlimb size, hindlimb size and tail (Figs. 2B, C, D).

Histological studies through abdominal regions were carried out to determine the anatomical defects. The selected liver sections from the control showed well developed spinal cord, diaphragm, liver lobes, lobar interzones and hepatic veins (Fig. 3A). The liver section from the vehicle control also showed well-formed spinal cord, lobes of liver and hepatic veins (Fig. 3B). The selected sections of liver from all dose groups showed necrosis and underdeveloped liver lobes (Figs. 3C, D, E). The sections from the antidote group showed well developed liver in all dose groups (Figs. 3F, G, H). The sections through heart and lungs of control and vehicle control showed well developed and well-formed heart and lungs (Figs. 4A, B). All dose groups showed anomalies like hypo pericardium, necrosis in accessory lobe of lung, underdeveloped middle lobe of lung, microcardia and necrosis in atrium (Figs. 4C, D, E). The sections through heart and lungs of antidote groups showed well developed and fully grown organs (Figs. 4F, G, H).

Skeleton preparations showed that well developed skeletal formation and normal ossification occurred in control fetuses (Fig. 5A) and in vehicle control fetuses (Fig. 6A). In dose group 6.25 µg/g B.W almost all fetuses were observed to have little to no ossification of the skeleton (Fig. 5B). In dose group 12.5 µg/g B.W,

fetuses showed slightly less ossification in limbs and tail region (Fig. 5C). The medium dose group showed less ossification as compared to low dose group. In dose group 25.0 µg/g B.W almost all fetuses with unossified skeletons were observed (Fig. 5D). The skeletons of fetuses from antidote groups showed normal levels of ossification, similar to control and vehicle control group (Figs. 6B, C, D).

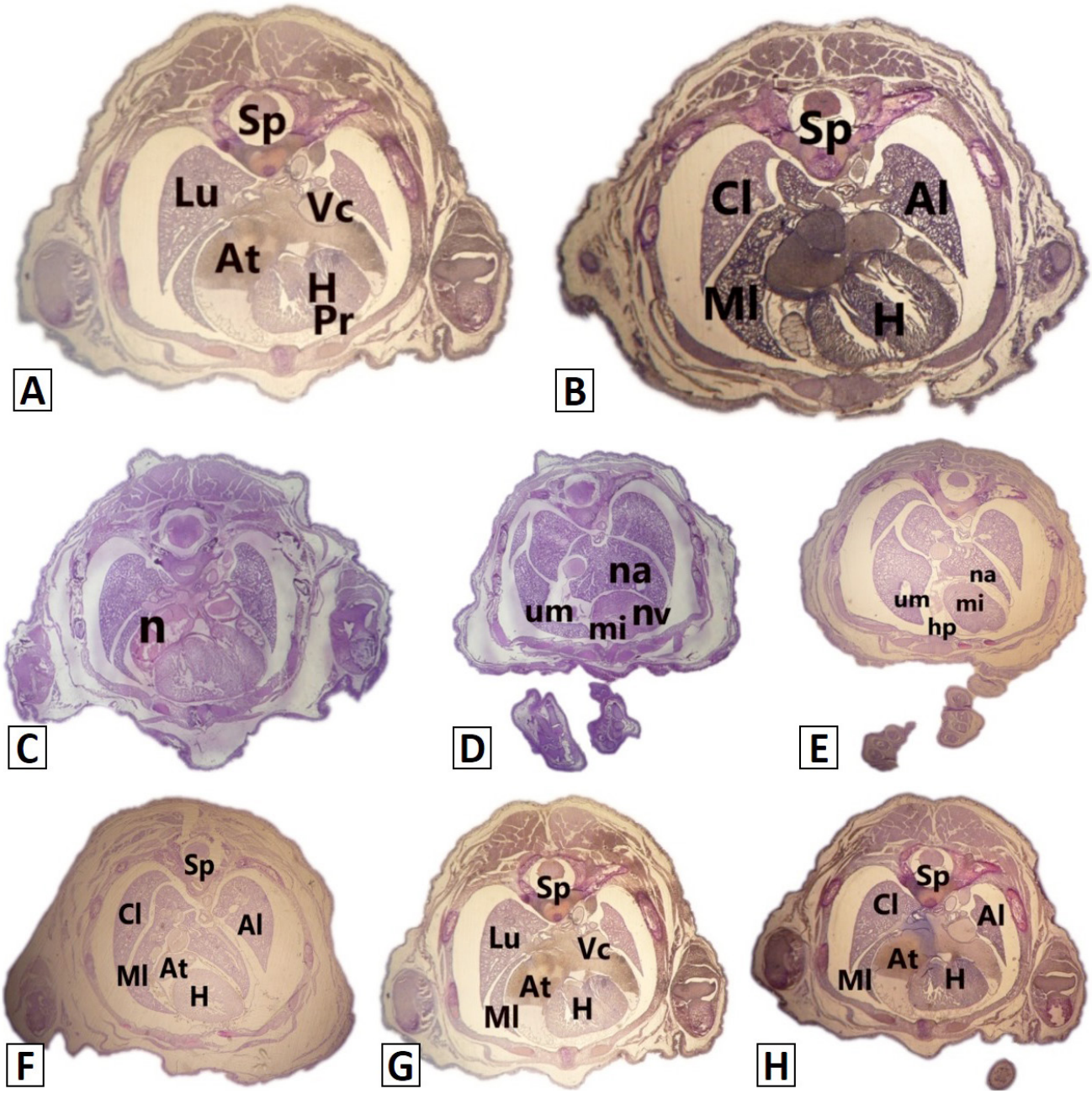

Fig. 3. Microphotographs of transverse sections of 18-day-old mouse through liver region fetuses recovered from mothers exposed to different doses of Cadmium chloride and cadmium +antidote on days 6 to 12 of gestation. **A,** control; **B,** vehicle control; **C,** 6.25 µg/g B.W; **D,** from 12.5 µg/g BW; **E,** from 25.0 µg/g BW; **F,** from antidote +6.25 µg/g BW; **G,** from antidote + 12.5 µg/g BW; **H,** from antidote+ 25.0µg/g BW. Ml, middle lobe of right lung; Cl, caudal lobe; Al, accessory lobe; At, lumen of right atrium; H, heart; Sp, spinal cord; Vc, vena cava; Pr, pericardial cavity; um, underdeveloped middle lobe; nv, left ventricle is underdeveloped; mi, microcardia; na, necrosis in accessory lobe of lung; n, necrosis in atrium; hp, hypo pericardium.

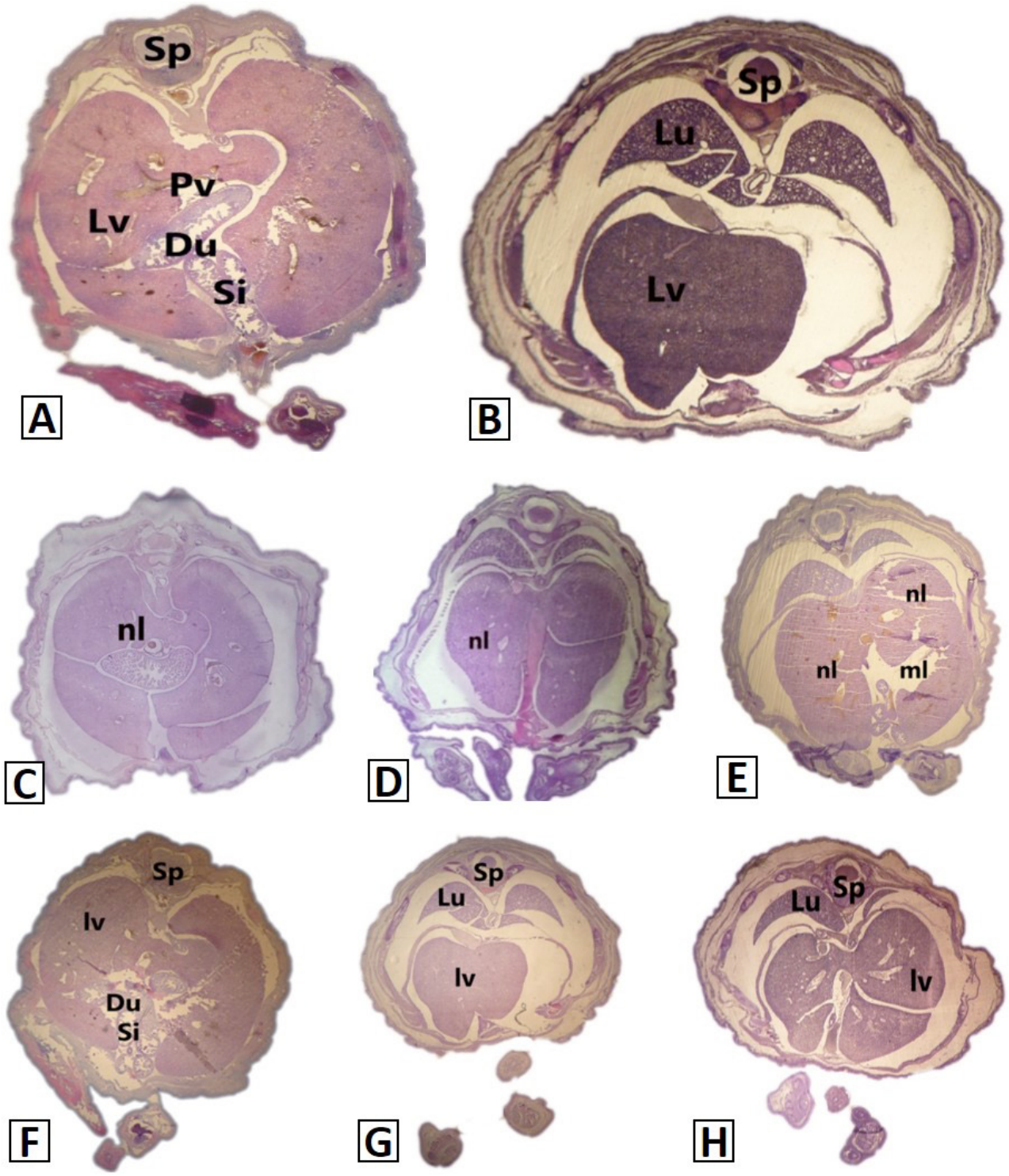

Fig. 4. Microphotographs of transverse sections of 18-day-old mouse through liver region fetuses recovered from mothers exposed to different doses of Cadmium and cadmium +antidote on days 6 to 12 of gestation. **A,** control; **B,** vehicle control; **C,** 6.25 µg/g B.W; **D,** from 12.5 µg/g BW; **E,** from 25.0 µg/g BW; **F,** from antidote +6.25 µg/g BW; **G,** from antidote + 12.5 µg/g BW; **H,** from antidote+ 25.0µg/g BW. Sp, Spinal cord; Lu, lung; lv, liver; Du, duodenum; Si, loops of small intestine; ml, small portion of left lobe is missing; nl, necrosis.

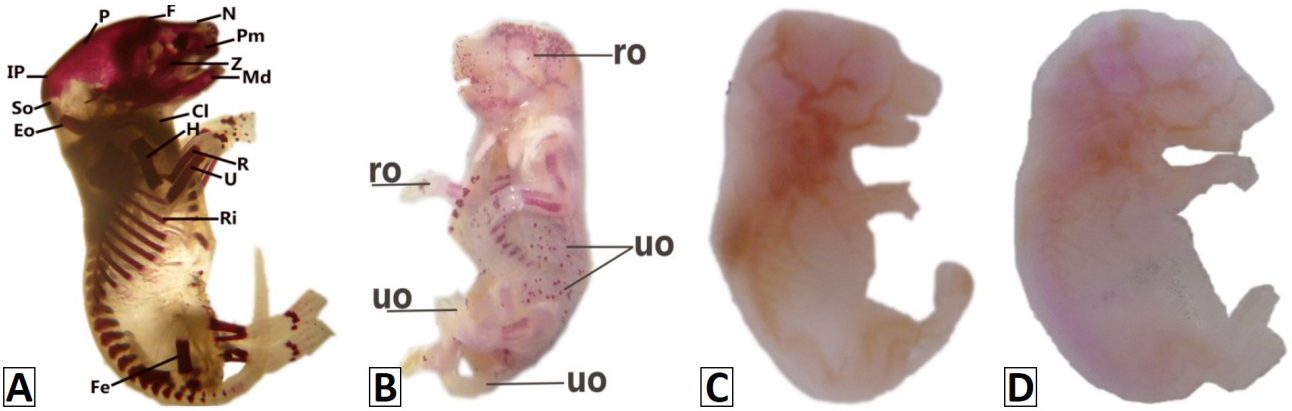

Fig. 5. **A,** control skeleton showing well ossified skeleton; **B,** from 6.25 µg/g BW; **C,** from 12.5 µg/g BW; **D,** from 25.0 µg/g BW. Cl, Clavical; Eo, Exoccipital; Fe, Femur; Fi, Fibula; F, Frontal; Ri, Ribs; U, Ulna; N, Nasal; Pm, Premaxilla; Z, Zygomatic; H, Humerus; So, Supraoccipital; Ip, Interparital; P, Parietal; R, Radius; Ro, Reduced ossification; Uo, Unossifie.

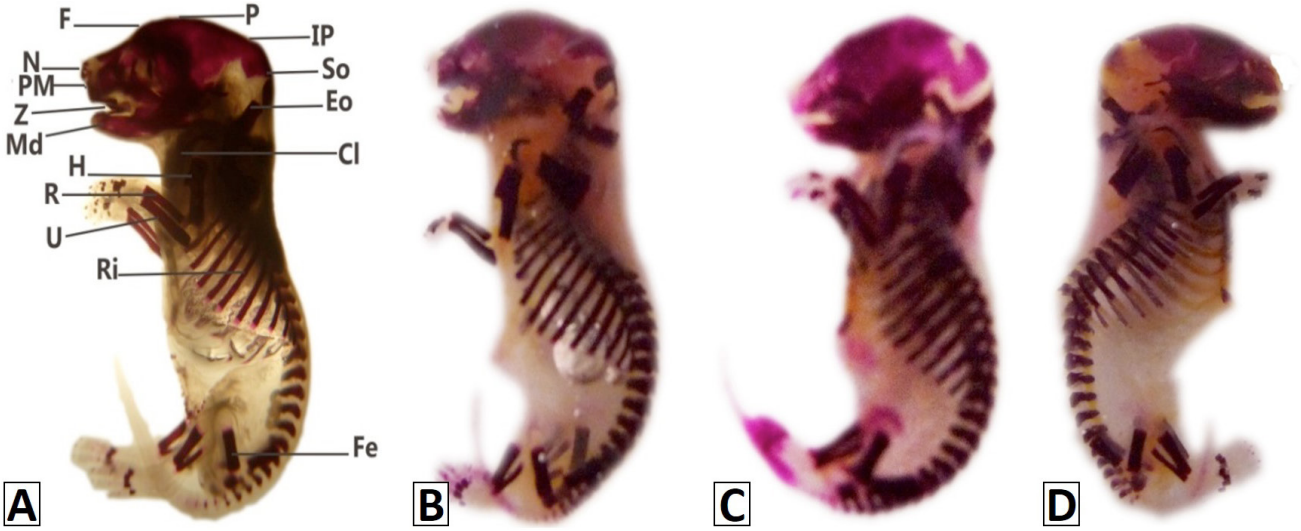

Fig. 6. Macrophotographs of fetal skeletal preparations of 18-day-old mouse fetuses recovered from mothers exposed to different doses of Cadmium or antidote on days 6 to 12 of gestation. **A,** vehicle control; **B,** from antidote +6.25 µg/g BW; **C,** from antidote + 12.5 µg/g BW; **D,** from antidote+ 25.0µg/g BW. For abbreviations, see Figure 5.

DISCUSSION

The present study that was designed to evaluate the disaster of cadmium and therapeutic effect of guava showed following results. Morphometric analysis showed a decrease in lengths of different structural parameters and increased in number of resorbtions that are in accordance to the study of Menai *et al*. (2012), where they examined pregnant mice that were given different doses of cadmium and reported the occurrence of growth retardation.

Limbs defects such as micromelia, distorted limbs, polydactyly, in this study were well supported by the findings of Messerle and Webster (1982). They injected the pregnant mice with 4mg/kg cadmium on days 8-10 days of gestation. There is a preaxial and postaxial reduction in limbs that occurred. These reductions cause limb anomalies such as syndactyly and polydactyly. In a study conducted by Elsaid *et al*. (2010), depict that cadmium chloride induce change in p53 gene which cause polydactyly and erectodactyly. Alexander *et al*. (2016) reported that limb abnormalities produced by cadmium might be the result of disruption in intracellular signaling pathways by interrupting cytoskeleton actin network. Consequently, cell adhesion, proliferation, migration and differentiation is disturbed causing limb anomalies in mice and rats model. But the supplementation with different

natural or synthetic materials can normalize the cell interaction and can reduce the chances of limb anomalies.

Neurological defects including open eyelids and exencephaly were observed in this study. It was similar to the studies by Castro *et al*. (2007) and Hsieh *et al*. (2009). It was proposed in their study that during neurulation cadmium exposure not only affected the structure but also the chemistry of the cells and produces endogenous marker signals reactive oxygen species (ROS) that cause the oxidative stress.

Along with a number of fetal morphological defects, histopathological anomalies of brain found in this study were dilations and absence of ventricles and underdeveloped cerebellum, which were supported by the study of Sun *et al*. (2005). They reported the cadmium caused neuronal cell apoptosis and necrosis. Sections through the cardiac region showed the abnormalities microcardia, necrosis in atrium, underdeveloped ventricles, hydroparicardium and lung abnormalities including underdeveloped lobes of lungs. Which were also observed by Yang *et al*. (1997). Gheorghescu and Thompson (2016) reported that in chick embryo, alteration in Ang-2 and VE cadherin cause delayed vasculogenesis and impaired angiogenesis. They described cadmium induced oxidative cellular damage in fetal lungs cells. Histopathological anomalies of liver found in this study were necrosis which was supported by Hideaki *et al*. (2004) and Fernandez *et al*. (2003). They studied that cadmium is accumulated in the liver and causes necrosis and hepatotoxicity. In common guava, morin is an active element. Studies showed that Morin-hydrate perform as an antioxidant that can defend rat liver cells against oxyradical damage (Kok *et al*., 2000). By decreasing the escape of enzymes into blood stream, morin can stabilize the hepatic cellular membrane and guard the hepatocytes against lethal effects of cadmium. This can be ascribed by the antioxidant property of Morin, which is the active ingredient of guava extract (Ayodele *et al*., 2013).

Furthermore, skeletal preparation showed reduced ossification in this study which were in accordance to study of Sughis *et al*. (2001). They found that cadmium in bone may interfere with whole bone mineralization process involving steps respectively calcification, decalcification and bone remodeling. Vitamins also prevent skeletal abnormalities. Normal skeletal formation is occurred in all the fetuses recovered from antidote group. In an experiment, Cahu *et al*. (2003) proved that nutritional deficiency induced skeletal abnormalities.

The fetuses recovered from the groups in which guava juice was given to the pregnant mice along with the different doses of cadmium, showed complete and normal development. The morphological, histological, morphometric and skeletal analysis of these fetuses showed that they have normal development of internal and external organs, completely ossified skeletons because of the ameliorative potential of the antioxidants and ascorbic acid in detoxifying the toxicity of heavy metal cadmium. All the results covered from guava fruit extract+cadmium doses supported by the following studies.

Guava is an excellent source of antioxidants so it has the ability to detoxify the cadmium and prevent the developmental anomalies induced by the cadmium. Jiménez *et al*. (2001) checked the antioxidant activity of guava fruit by using three different complementary methods. The entire test groups showed that guava has undeniable antioxidant activity and this activity of guava is depended upon the phenolic concentrations of fruit. Besides guava, selenium can also ameliorate the toxic effects of cadmium but among guava and selenium, guava had more therapeutic values that is why guava was used in this study.

CONCLUSION

It is concluded by this study, guava that is rich in antioxidants, vitamin C, vitamin A and phenols, can exert a therapeutic effect on embryo toxicity and anomalies induced by prenatal cadmium exposure in developing mice.

Statement of conflict of interest

Authors have declared no conflict of interest.

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