ORIGINAL ARTICLE

Veterinary Research Forum. 2025; 16 (4): 203 - 208
doi: 10.30466/vrf.2024.2031444.4313
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Determination of the effect of *Tarantula cubensis* alcoholic extract on cadmium embryotoxicity

Rahmi Canbar[1], Gokhan Akcakavak[2]*, Muhittin Uslu[3], Mustafa Sedat Arslan[4], Harun Kizilay[5]

[1] Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Necmettin Erbakan University, Konya, Türkiye; [2] Department of Pathology, Faculty of Veterinary Medicine, Aksaray University, Aksaray, Türkiye; [3] Department of Laboratory and Veterinary Health, Sefaatli Vocational College, Yozgat Bozok University, Yozgat, Türkiye; [4] Prof. Dr. Humeyra Ozgen Research and Application Farm, Faculty of Veterinary Medicine, Selcuk University, Konya, Türkiye; [5] Department of Pharmacology, Faculty of Pharmacy, Selcuk University, Konya, Türkiye.

Article Info

Article history:

Received: 13 June 2024
Accepted: 17 July 2024
Available online: 15 April 2025

Keywords:

Tarantula cubensis alcoholic extract
Cadmium
Embryotoxic
in ovo methods

Abstract

Tarantula cubensis alcoholic extract (TCAE) is a homeopathic product used in the veterinary field. This study aimed to determine the effects of TCAE on cadmium (Cd) toxicity in the embryo. The study used 220 fertile, incubated chicken eggs divided into 11 equal groups on the 7th day of incubation. The groups comprised untreated and physiological saline control groups, a group with TCAE alone, four groups with varying doses of Cd alone and four groups with the same doses of Cd plus TCAE. At the end of the incubation period, the eggs were opened, kidney and liver tissue samples were taken for histopathology and the number of dead and living embryos were recorded. In the prsent study, the median lethal dose of Cd was determined to be 0.029 mg *per* egg and the median lethal dose of Cd plus TCAE was determined to be 0.020 mg *per* egg. The histopathological examinations determined that kidney and liver damage were increased when TCAE and Cd were administered together, that was higher than when Cd was given alone. Thus, TCAE, which had no toxic effect on the embryo when used alone, might increase the embryotoxic activity of Cd. However, more detailed studies are needed.

Introduction

Cadmium (Cd) is an environmental pollutant with teratogenic and carcinogenic effects,[1] and a half-life of 25 - 30 years in plants and animals.[2] The Cd may disrupt placental function and affect fetal development by passing through the placenta or accumulating in placenta during pregnancy.[3] The Cd toxicity in chicks disrupts peridermal cell adhesion and causes cell death in the mesoderm which may lead to abnormal growth of the mesoderm and body wall defect.[4] Another study found a correlation between the formation of ventral body wall defects due to Cd and the apoptosis occurring in the somites, lateral plate mesoderm and neural tube. The degree of apoptosis is correlated with the degree of peridermal destruction.[5]

Tarantula cubensis alcoholic extract (TCAE) is a homeopathic product used in the veterinary field, generally for the treatment of conditions such as gangrene, septicemia and toxemia.[6] It accelerates the healing of oral lesions[7] and the repair of tendon ruptures.[8] In a study conducted on sheep, it showed an antioxidant effect,[9] caused cancer cells to undergo apoptosis via the caspase-3 pathway in vitro[10] and had clinically positive effects on mammary tumors in dogs[11] through apoptosis.[12] Although its mechanism is not clearly defined, it has reduced aberrant crypt foci and polyp formation in experimental colon cancer.[13-15] TCAE has also been reported to have a decreasing effect on cell proliferation markers in colon cancer.[16] Moreover, it has been reported to have a reducing effect on experimentally induced hepatocellular carcinoma tumor morphology.[17] Although there is limited information on the use of TCAE during pregnancy, it is stated to be safe in *in ovo* study.[18]

It has been reported that the heavy metal Cd has an embryotoxic effect and can be used in modeling experimental animals in ventral wall defect studies.[4,5,19] The TCAE has been found to have no direct embryotoxic activity on fertile chicken eggs.[18] Additionally, no studies have been found evaluating the effects of TCAE which has been shown to have anti-inflammatory, antioxidant and antitumor effects in different studies,[9,10,13] on Cd toxicity.

The present study hypothesized whether TCAE had any activity on embryotoxicity when used in combination with embryotoxic Cd.

***Correspondence:**

Gokhan Akcakavak. PhD
Department of Pathology, Faculty of Veterinary Medicine, Aksaray University, Aksaray, Türkiye
E-mail:gokhan.akcakavak@aksaray.edu.tr

Materials and Methods

Materials. In this research, fertile chicken eggs obtained from a commercial enterprise completed their incubation period in an incubator (Imza Teknik, Konya, Türkiye). On the 7th day of incubation, non-fertile eggs checked for fertility under light were removed from the groups and fertile eggs were replaced keeping each group at 20. On the 7th day, injection into the air chambers of all eggs in the study was performed. Pure Cd chloride (Carlo Erba, Val-de-Reuil, France) was dissolved in physiological saline. The study protocol was approved by the local ethics committee, Selçuk University Faculty of Veterinary Medicine, Experimental Animal Production and Research Center Ethics Committee, Number 2023/102.

Experimental design and animal applications. In the first phase of the study, 220 fertile eggs were divided into 11 groups on the 7th day of incubation, with 20 fertile eggs in each group and incubated. The groups consisted of (1) untreated control group, (2) physiological saline control 70.00 µL *per* egg, (3, 4, 5, 6) Cd at doses of 0.01, 0.02, 0.04, and 0.08 mg *per* egg, respectively, administered in a volume of 20.00 µL *per* egg, (7) TCAE, applied at a dose of 50.00 µL *per* egg, (8, 9, 10, 11) TCAE 50.00 µL *per egg* plus Cd at a dose of 0.01, 0.02, 0.04, and 0.08 mg *per* egg, respectively, in a volume of 20.00 µL *per* egg.

Histopathological examination. After the incubation period (21 days), the eggs were opened, the number of live and dead embryos was recorded and tissue samples were taken for histopathological examination. Liver and kidney tissues taken from chicks were fixed in 10% a neutral formaldehyde solution for 24 - 48 hr. Routine tissue follow-up procedures were performed for histopathological examination. Sections (4.00 -5.00 µm thickness) were taken from the obtained paraffin blocks and stained with Hematoxylin and Eosin.[15,16] Histopathological changes were evaluated in 10 different areas under a light microscope (BX51; Olympus, Tokyo, Japan) at 20× magnification as (-): No lesions, (+): Mild lesions, (++): Moderate lesions and (+++): Severe lesions.

Statistical analysis. In this study, the actual mortality rate was determined with the Abbott formula.[20,21]

$$Abbott\ formula = \frac{Actual\ mortality\ rate - provisional\ mortality\ rate}{100 - provisional\ mortality\ rate}$$

Intergroup survival rates were evaluated with the chi-square test. The median lethal dose (LD_{50}) level was determined by the probit method (version 22.0; SPSS Inc., Chicago, USA). A $p < 0.05$ value was considered statistically significant.

Results

Determination of dead and alive numbers and LD_{50}. Information on the numbers of living and dead embryos in the study is given in Table 1. A significant statistical dose-dependent difference was seen in the mortality rates among groups. When examined by probit analysis in the study, the LD_{50} of Cd alone was determined as 0.029 (0.015 - 0.037) mg *per* egg. This was decreased with the application of TCAE, calculated as 0.020 (0.06 - 0.028) mg *per* egg.

Histopathological results. Six surviving chicks were randomly selected for histopathology. However, as there were three survivors in the 0.04 Cd+TCAE group, the evaluation was made on three animals. Also, it was observed that all the animals in the 0.08 Cd+TCAE and 0.08 Cd groups died. Scores among groups are presented in Table 2. Histopathological examination showed that the liver and kidney tissues of the control group embryos had normal histological structure. Changes were absent and/or very mild in the TCAE group. Other experimental groups (Cd and Cd+TCAE) showed moderate to severe necrotic changes, congestion and sinusoidal dilatation in the liver. Significant histopathological changes were detected in the kidney including moderate to severe necrotic changes in tubular epithelium, congestion, tubular dilatation and widening of the Bowman space (Fig. 1). The highest scores among the groups were generally found with 0.04 Cd and 0.04 Cd+TCAE.

Table 1. Dead and alive rates in embryos.

Groups	Alive embryos	Dead embryos	Total death (%)	Abbott formula[20,21]
Control	19	1	5.00^{e}	-
CSF	18	2	10.00^{e}	-
0.01 Cd	18	2	10.00^{e}	0.00
0.02 Cd	12	8	40.00^{d}	33.30
0.04 Cd	5	15	75.00^{bc}	72.20
0.08 Cd	0	20	100^{a}	100
TCAE	18	2	10.00^{e}	0.00
0.01 Cd+TCAE	13	7	35.00^{d}	27.70
0.02 Cd+TCAE	9	11	55.00^{cd}	50.00
0.04 Cd+TCAE	3	17	85.00^{ab}	83.30
0.08 Cd+TCAE	0	20	100^{a}	100

CSF: Physiological saline control, Cd: Cadmium, and TCAE: *Tarantula cubensis* alcoholic extract.
$^{a-e}$ Different letters in the same column are statistically different (Chi-square test, $p < 0.05$).

Table 2. Histopathological scores in experimental groups.

Changes	Control	CSF	TCAE	0.01 Cd	0.02 Cd	0.04 Cd	0.01 Cd+TCAE	0.02 Cd+TCAE	0.04 Cd+TCAE
Number of samples	**6**	**6**	**6**	**6**	**6**	**5**	**6**	**6**	**3**
Liver									
Necrotic changes hepatocytes	-	-	-	++	++	++	++	++	+++
Congestion	-	-	+	++	++	+++	++	++	+++
Sinusoidal dilation	-	-	+	++	++	+++	++	++	+++
Kidney									
Necrotic changes in tubular epithelium	-	-	-	++	++	++	++	++	+++
Congestion	-	-	+	++	++	+++	++	+++	+++
Tubular dilation	-	-	-	++	++	+++	++	+++	+++
Bowman's space expansion	-	-	+	++	++	+++	++	+++	+++

CSF: Physiological saline control, Cd: Cadmium, and TCAE: *Tarantula cubensis* alcoholic extract.
(-): No lesions, (+): Mild lesions, (++): Moderate lesions, (+++): Severe lesions.

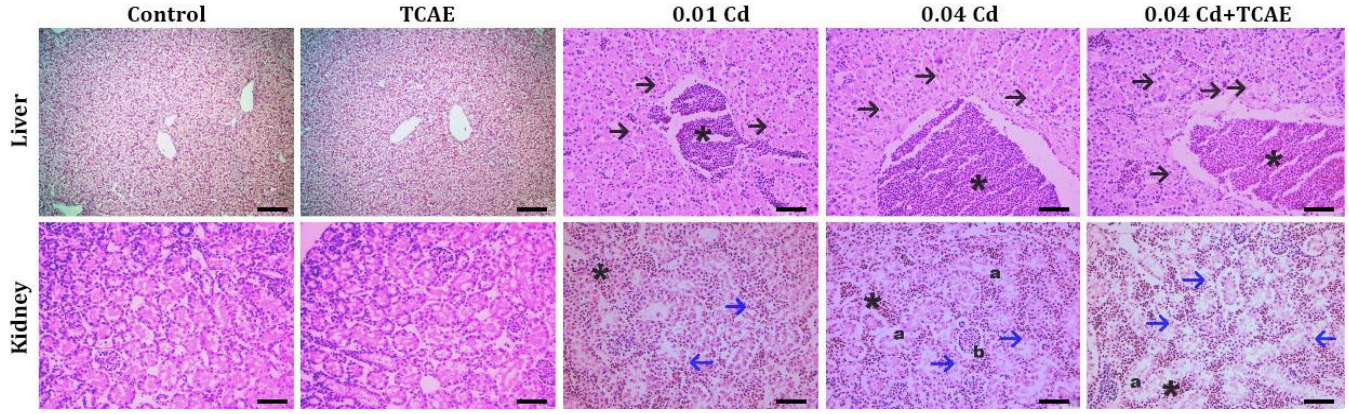

Fig. 1. Microscopic view of histopathological changes in groups including control, *Tarantula cubensis* alcoholic extract (TCAE) and Cadmium (Cd) showing congestion (asterisk), necrotic changes in hepatocytes (black arrows), necrotic changes in kidney tubules (blue arrows), tubular dilatation (a) and expansion of bowman's space (b). (Hematoxylin-Eosin staining; Bars in liver control and TCAE = 100 µm, in the rest = 50.00 µm).

Discussion

In addition to teratogenic and carcinogenic effects, Cd is known to have embryotoxic effects.[1] Literature states that TCAE, which is used as a homeopathic product for the treatment of many pathological conditions in veterinary medicine,[6] does not have embryotoxic effects.[18] Present study evaluated the effectiveness of TCAE on Cd embryotoxicity.

This study observed that Cd application at 0.020 mg *per* egg and higher doses had a positive correlation with embryotoxicity (Table 1, $p < 0.05$). The embryotoxic LD_{50} of Cd was determined t obe 0.029 mg *per* egg. It has been found that maternal Cd exposure in the mid-pregnancy phase (unlike the early pregnancy phase) in humans increases the risk of a smaller-than-normal fetus.[22] It is reported that maternal Cd exposure in the early stages of pregnancy is inversely associated with birth weight and ponderal index in female, but not male, infants.[23,24]

The total six major categories of developmental abnormalities have been observed in zebrafish embryos with Cd toxicity including head and eye hypoplasia, hypopigmentation, cardiac edema, yolk sac deformities, altered axial curvature and tail malformations.[25] It has been found that exposure to Cd during the gastrulation period in zebrafish embryos causes formation of abnormal somites in muscle fibers, defects in axonogenesis and failure of the notochord to extend to the tail region.[26] The Cd has effects on neural tube closure, limb development and soft tissue formation in rodents dependent on the time of application.[27] The authors regarded this as a reliable exomphalos animal model of ventral body wall defect due to Cd toxicity. A positive relationship between exomphalos and lumbar lordosis has also been reported.[19] Yamamoto *et al.*[28] found that Cd at a dose of 50.00 µM had teratogenic effects in the early stages of chicken development (stages 12 to 19) causing critical structures to fail to develop especially in the cephalic region. The ventral body wall defect of Cd application in chick embryos has been attributed to changes in the somites following the interruption of a signaling pathway originating from the ectoderm.[5] It has also been reported that Cd application increased the malondialdehyde level in chick embryos and caused DNA damage at doses of 40.00 and 60.00 µg *per* egg.[29] Our study found a positive correlation between the amount of Cd applied to embryos and embryotoxicity similar to the cited literature.

The TCAE is a homeopathic product used in veterinary medicine. In this study, 0.01, 0.02, 0.04, and 0.08 mg *per*

egg doses of Cd cadmium and 50.00 µL *per* egg TCAE were applied together to the air space of fertile chicken eggs and their incubation period (21 days) was completed. The study determined that TCAE was not embryotoxic when used alone, however, the embryotoxicity of Cd was increased when used with TCAE (Table 1, $p < 0.05$). The LD_{50} of Cd when used with TCAE was 0.020 mg *per* egg compared to 0.029 mg *per* egg when used alone.

Studies on the safety or embryotoxicity of homeopathic products during pregnancy are limited. The literature lacks sufficient information on the safety of TCAE use during pregnancy. However, it is stated that TCAE alone is not embryotoxic at different doses.[18] Studies have found that selenium, manganese and nickel may be protective against Cd toxicity, however, calcium, magnesium, and verapamil do not have a protective effect.[30] The use of homeopathic medicines is recommended in humans to prevent problems that occur during pregnancy or the postpartum period (anemia, nausea, pica, breech presentation, mastitis, prolonged pregnancy and placental retention).[31,32] It is stated that there is no difference in the frequency of adverse effects between homeopathic treatment and placebo treatment. It is stated that the resulting side effects are not related to the treatment, but to the patient's condition.[33] It was stated that Good Manufacturing Practices (GMP) and International Organizarion for Standardization (ISO) certified Argenticum metalum, Calcarea carbonica and Sepia were not embryotoxic in the study conducted on zebrafish.[34] It is also stated that homeopathic products like Sepia and Nux Vomica (30 C dilution) did not have a toxic effect on mouse embryonic stem cells.[35] In a study conducted on broiler chickens, it was reported that bee venom (0.50, 1.00, and 1.50 mg) did not have negative effects on the productivity performance and physiology of chickens.[36] In the study conducted on rats, it was stated that the ethanolic extract of Aegialitis rotundifolia did not cause pathological lesions other than its sedative effect at low doses, however, a few minor changes were observed in rats at high doses. It is also stated that it could be used safely in low doses.[37] It is reported that homeopathic products like Ferrum phosphoricum 3X, Ferrum phosphoricum 6X, Calcarea phosphoricum 6X, and Magnesium phosphoricum 6X did not have toxic effects on rats.[38] It has also been reported that spider (Nephila clavata, Araneus ventricosus) venom products may have insecticidal effects,[39,40] however, have no effect on vertebrates.[39] As a result, it could be stated that TCAE was not embryotoxic on its own, but when used together with Cd, which is known to be embryotoxic, it could increase the embryotoxic effect of Cd.

Experimental studies conducted with Cd in birds report that high amounts of Cd bioaccumulate, especially in the kidney and liver tissues and cause serious histopathological damage in the relevant tissues.[41-43] In different studies, it has been reported that histopathological findings such as degenerative and necrotic changes, congestion, tubular dilatation and hyaline cast were detected in the renal tubules in association with Cd.[41,44] Andleeb *et al.*[45] reported in their study that they detected necrotic changes (pyknosis) and steatosis in the livers of embryos after treatment with Cd chloride at a dose of 1.50 µg *per* 0.05 mL *per* egg. Kedam *et al.*[46] reported that in their study using Cd chloride, they detected histopathological sinusoidal dilatation, necrotic changes and bleeding in the liver of chick embryos. In the current study, histopathologically, hepatocytes necrotic changes, congestion and sinusoidal dilatation in the liver, necrotic changes in the tubules in the kidney, congestion, tubular dilatation and widening of the Bowman space were detected (Fig. 1). The current findings confirmed embryotoxicity and were in agreement with the findings of previous studies. In addition, microscopic findings showed that the combined use of Cd and TCAE increased the severity of necrotic changes in hepatocytes and tubules. Thus, it could be stated that histopathologically TCAE had an increasing effect on Cd embryotoxicity.

Previously, Canbar *et al.*[18] reported that TCAE had no effect on embryotoxicity. In this context, the focus of our study was to evaluate the effectiveness of TCAE, a homeopathic product with many medical effects such as anti-inflammatory, antioxidant and anti-apoptotic, on Cd embryotoxicity. The results of the current study revealed that TCAE showed Cd embryotoxicity enhancing properties. Considering the results of the present study, we suggest that future studies investigate the effects of TCAE on Cd embryotoxicity at the molecular level. Thus, it would provide a more comprehensive perspective on the mechanism of action.In conclusion, in the present study, it was observed that TCAE did not have a toxic effect on embryos, but when applied with cadmium, which has a known toxic effect, TCAE enhanced toxic activity, increased liver and kidney damage, as shown by histopathological examination, and increased the number of dead embryos. More molecular studies are needed in the future to elucidate the embryotoxicity-enhancing effect of TCAE.

Acknowledgments

The abstract of this article is presented at 2nd International Congress of Multidisciplinary Medical and Health Sciences Studies, held during 27-28 May 2024, Ankara, Türkiye. We would like to thank Selçuk University BAP Unit, Konya, Türkiye, for its financial support in the realization of the project, (Project No. 23401110).

Conflict of interest

There is no conflict of interest between the authors.

References

1. Kayhan FE. Bioaccumulation and toxicity of cadmium in aquaculture [Turkish]. Ege J Fish Aquat Sci 2006; 23(1-2): 215-220.
2. Genchi G, Sinicropi MS, Lauria G, et al.The effects of cadmium toxicity. Int J Environ Res Public Health 2020; 17(11): 3782. doi: 10.3390/ijerph17113782.
3. Geng HX, Wang L. Cadmium: toxic effects on placental and embryonic development. Environ Toxicol Pharmacol 2019; 67: 102-107.
4. Thompson J, Bannigan J. Effects of cadmium on formation of the ventral body wall in chick embryos and their prevention by zinc pretreatment. Teratology 2001; 64(2): 87-97.
5. Thompson J, Hipwell E, Loo HV, et al. Effects of cadmium on cell death and cell proliferation in chick embryos. Repro Toxicol 2005; 20(4): 539-548.
6. Richardson-Boedler C. The brown spider Loxosceles laeta: source of the remedy Tarentula cubensis? Homeopathy 2002; 91(3): 166-170.
7. Albay MK, Şahinduran Ş, Kale M, et al. Influence of Tarantula cubensis extract on the treatment of the oral lesions in cattle with bluetongue disease. Kafkas Univ Vet Fak Derg 2010; 16(4): 593-596.
8. Bonamin LV, Cardoso TN, de Carvalho AC, et al. The use of animal models in homeopathic research - - a review of 2010-2014 PubMed indexed papers. Homeopathy 2015; 104(04): 283-291. doi: 10.1016/j.homp.2015.06.002.
9. Dik B, Er A, Çorum O. Effect of Tarantula cubensis alcoholic extract (Theranekron®) on serum thiobarbituric acid reactive products in sheep [Turkish]. Eurasian J Vet Sci. 2014; 30(2): 68-71.
10. Ghasemi-Dizgah A, Nami B, Amirmozafari N. Tarantula cubensis venom (Theranekron®) selectively destroys human cancer cells via activating caspase-3-mediated apoptosis. Acta Med Int 2017; 4(1): 73-79.
11. Gültiken N, Vural MR. The effect of Tarantula cubensis extract applied in pre and postoperative period of canine mammary tumours. J Istanbul Vet Sci 2007; 2: 13-23.
12. Gultiken N, Guvenc T, Kaya D, et al. Tarantula cubensis extract alters the degree of apoptosis and mitosis in canine mammary adenocarcinomas. J Vet Sci 2015; 16(2): 213-219.
13. Er A, Ozdemir O, Coskun D, et al. Effects of Tarantula cubensis alcoholic extract and Nerium oleander distillate on experimentally induced colon cancer. Revue Méd Vét 2019; 170(1-3): 15-21.
14. Akcakavak G, Ozdemır O. Effect of Tarantula cubensis alcoholic extract on tumour pathways in azoxy-methane-induced colorectal cancer in rats. Acta Vet Brno 2023; 92: 79-88.
15. Akcakavak G, Celik Z, Karatas O, et al. Tarantula cubensis alcohol extract enhances the tumoricidal effect of capecitabine via multiple pathways in azoxymethane-induced colorectal cancer in rats. Trop J Pharm Res 2024; 23(2): 291-297.
16. Ozdemir O, Akcakavak G, Tuzcu M. Effect of Tarantula cubensis alcoholic extract and Nerium oleander distillate on cell proliferation markers in colon carcinogenesis. Rev Cient Fac Cienc Vet Univ Zulia 2022; 32: e32150. doi: 10.52973/rcfcv-e32150.
17. Vanli S, Kurtoglu F, Alan BS, et al. Investigation of the effects of Theranekron and Sorafenib treatments on carcinogenesis, apoptosis and biochemical profile in hepatocellular carcinoma in rats. Toxicol Mech Methods 2024; 34(7): 750-760.
18. Canbar R, Akcakavak G, Uslu M, et al. Determination of embrvotoxic effects of Tarantula cubensis alcoholic extract with *in ovo* model. Magy Allatorvosok Lapja 2021; 143(8): 497-504.
19. Thompson JM, Bannigan JG. Omphalocele induction in the chick embryo by administration of cadmium. J Pediatr Surg 2007; 42(10): 1703-1709.
20. Uslu M, Canbar R, Arslan MS, et al. Determination of the embryotoxic effect of metronidazole using an in ovo model. Rev Cient Fac Cienc Vet Univ Zulia 2024; 34: rcfcv-e34310. doi: 10.52973/rcfcv-e34310.
21. Dayan MO, Canbar R, Besoluk K, et al. Determınıng LD50 and teratogenıc effects of marbofloxacın ın *ın ovo* model. Fresenius Environ Bull 2022**;** 31(08B/2022): 8673-8679.
22. Wang H, Liu L, Hu YF, et al. Maternal serum cadmium level during pregnancy and its association with small for gestational age infants: a population-based birth cohort study. Sci Rep 2016; 6: 22631. doi: 10.1038/srep22631.
23. Taylor CM, Golding J, Emond AM. Moderate prenatal cadmium exposure and adverse birth outcomes: a role for sex-specific differences? Paediatr Perinat Epidemiol 2016; 30(6): 603-611.
24. Cheng L, Zhang B, Zheng T, et al. Critical windows of prenatal exposure to cadmium and size at birth. Int J Environ Res Public Health 2017; 14(1): 58. doi: 10.3390/ijerph14010058.
25. Cheng SH, Wai AWK, So CH, et al. Cellular and molecular basis of cadmium-induced deformities in zebrafish embryos. Environ Toxicol Chem 2000; 19(12): 3024-3031.
26. Hen Chow ES, Cheng SH. Cadmium affects muscle type development and axon growth in zebrafish embryonic somitogenesis. Toxicol Sci 2003; 73(1): 149-159.
27. Thompson J, Bannigan J. Cadmium: toxic effects on the reproductive system and the embryo. Reprod toxicol 2008; 25(3): 304-315.
28. Yamamoto FY, Filipak Neto F, Freitas PF, et al. Cadmium effects on early development of chick embryos. Environ Toxicol Pharmacol 2012; 34(2): 548-555.

29. Meena Bai M, Divya K, Haseena Bhanu SK, et al. Evaluation of genotoxic and lipid peroxidation effect of cadmium in developing chick embryos. J Environ Anal Toxicol 2014; 4(6): 1000238. doi: 10.4172/2161-0525.1000238.
30. Cullinane J, Bannigan J, Thompson J. Cadmium teratogenesis in the chick: period of vulnerability using the early chick culture method, and prevention by divalent cations. Repro Toxicol 2009; 28(3): 335-341.
31. Katz T. The management of pregnancy and labour with homoeopathy. Complement Ther Nurs Midwifery 1995; 1(6): 159-164.
32. Steinberg D, Beal MW. Homeopathy and women's health care. J Obstet Gynecol Neonatal Nurs 2003; 32(2): 207-214.
33. Habs M, Koller M. Material risks of homeopathic medicinal products: regulatory frameworks, results of preclinical toxicology, and clinical meta-analyses and their implications. Complement Med Res 2021; 28(1): 64-84.
34. Gupta HR, Patil Y, Singh D, et al. Embryonic zebrafish model -A well-established method for rapidly assessing the toxicity of homeopathic drugs: toxicity evaluation of homeopathic drugs using zebrafish embryo model. J Pharmacopuncture 2016; 19(4): 319-328.
35. Jyoti S, Tandon S. Impact of homeopathic remedies on the expression of lineage differentiation genes: an in vitro approach using embryonic stem cells. Homeopathy 2016; 105(02): 148-159.
36. Ali AHH, Mohanny KM. Effect of injection with bee venom extract on productive performance and immune response of broiler chicks. J Anim Poult Prod 2014; 5(5): 237-246.
37. Ghosh D, Mondal S, Ramakrishna K. Acute and sub-acute (30-day) toxicity studies of *Aegialitis rotundifolia* Roxb., leaves extract in Wistar rats: safety assessment of a rare mangrove traditionally utilized as pain antidote. Clin Phytosci 2019; 5: 13. doi: 10.1186/s40816-019-0106-2.
38. Singh S, Kalra P, Karwasra R, et al. Safety studies of homoeopathic drugs in acute, sub‐acute and chronic toxicity in rats. Indian J Res Homoeopathy 2017; 11: 48-57.
39. Liu K, Wang M, Herzig V, et al. Venom from the spider Araneus ventricosus is lethal to insects but inactive in vertebrates. Toxicon 2016; 115: 63-69.
40. Jin L, Fang M, Chen M, et al. An insecticidal toxin from Nephila clavata spider venom. Amino acids 2017; 49(7): 1237-1245.
41. Akter MT, Ferdous KA, Rahaman T, et al. Exposure to environmental heavy metal (cadmium) through feed and its effect on bio-histomorphological changes in commercial quail. J Entomol Zool Stud 2019; 7(5): 965-971.
42. Cinar M, Yigit AA, Yalcinkaya I, et al. Cadmium induced changes on growth performance, some biochemical parameters and tissue in broilers: effects of vitamin C and vitamin E. Asian J Anim Vet Adv 2011; 6(9): 923-934.
43. Kar I, Patra AK. Tissue bioaccumulation and toxico-pathological effects of cadmium and its dietary amelioration in poultry - a review. Biol Trace Elem Res 2021; 199(10): 3846-3868.
44. Butt SL, Saleemi MK, Khan MZ, et al. Cadmium toxicity in female Japanese quail (Coturnix japonica) and its diminution with silymarin. Pak Vet J 2018; 38(3): 2018.062. doi: 10.29261/pakvetj/2018.062..
45. Andleeb S, Shaukat S, Ara C. Protection against cadmium-induced abnormalities and hepatotoxicity in ovo by Allium sativum. Punjab Univ J Zool 2018; 33(1): 34-41.
46. Kedam TR, Sheik RB, Bai MM, et al. A histological study on acrylamide and cadmium chloride altered chick embryonic liver. IOSR J Pharm 2012; 2(1): 1-8.