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TERATOGENIC EFFECT OF THE COUMARINIC ANTICOAGULANT RODENTICIDE, RACUMIN IN WHITE RATS

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ABSTRACT

The present study gives an overview of the in utero exposure of the developing rat fetus to Racumin rodenticide. In the current investigation, pregnant rats were exposed orally to either 1.65 or 0.83 mg/kg b.wt (1/10 and 1/20 LD_{50}, respectively) of coumatetralyl daily on days 6 through 15 of gestation (organogenesis period). Maternal and fetal parameters were evaluated on day 20 of gestation. Fetuses were weighed and examined for external, visceral and skeletal malformations. Exposure to this rodenticide at the two tested doses significantly increased the number of resorption and implantation sites (post implantation loss), dead fetuses' number per dam and placental weights. The number of live fetuses per dam and the fetal body weight were significantly reduced in the treated group at 1.65 mg/kg b.wt.when compared to the other treated and control groups.

Gross examination of the fetuses from Racumin treated dams' at both doses revealed significant incidences of dwarfism, subcutaneous hemorrhages, generalized subcutaneous edema and micrognathia. Racumin significantly increased the total fetuses with visceral and skeletal anomalies in a dose – dependent manner compared to the control. The major anomalies recorded were intrathoracic hemorrhage, hydrocephaly, heart and lung hypoplasia, anophthalmia, incomplete ossification of skull, sternebrae and coccygeal vertebrae. It could be concluded that Racumin has a dose – dependent teratogenic potential in rats.

Key Words: Anticoagulants – Rodenticides - Racumin - Coumatetralyl – Teratogenicity – Female – reproduction – rats.

INTRODUCTION

Anticoagulant rodenticides (Vitamin K antagonists) are widely used as the most effective method to control pest rodents. Several coumarin congeners beside warfarin (WF) were synthesized and used as anticoagulant rodenticides, including brodifacoum, bromadiolone, coumachlor, coumatetralyl, difenacoum (first generation hydroxycoumarins; Lund, 1988). The increased efficacy of these 4 – OH coumarins as rodenticides results from their high lipid solubility, affinity for hepatic tissue and extremely slow elimination from the body (Mosterd andThijssen, 1991). The 4-hydroxycoumarin anticoagulants as vitamin K antagonists are also used in prophylactic medicine to prevent thromboembolic diseases in patients at risk for nearly 50 years (Furie, 2000 and Osman *et al.*, 2005).

Anticoagulant rodenticides are often the cause of accidental poisoning of domestic animals. In addition, their use has the potential to cause environmental damage through poisoning of wildlife that feed on the baits or on rodents that have consumed and accumulated the poison in the body (Fisher *et al.*, 2003).

Percutaneous absorption of anticoagulant rodenticides represents a potential risk for workers and persons applying them. Several incidents of anticoagulant rodenticides intoxication via percutaneous route have been reported in the literature (Spiller *et al.*, 2003). In these reports, systemic effects and coagulopathies, occasionally with fatal outcome were described. In addition, many cases of oral intoxication both in humans (Morgan *et al.*, 1998) and animals (Marchhini and Turillazzi, 1978 and Twigg and Kay, 1995) had been reported.

Racumin (coumatetralyl) as one of the coumarinic anticoagulant rodenticides was found to be more toxic to Rattus norvegicus than the indane-dione anticoagulant rodenticides (Diphacinone and chlorophacinone) and considered a good alternative to warfarin (Fauconnet *et al.*, 1997). It is classified by the WHO as a highly dangerous chemical (WHO, 1995). In rats, the acute oral LD_{50} of Racumin (coumatetralyl) is 16.5 mg/kg b.wt. However, the sub chronic oral LD_{50} (5 days) is 0.3 mg/kg b.wt./day (Tomlin, 1994).

From the literature, only rare cases of human fetal anomalies were recorded regarding the developmental

toxicity of coumarins especially warfarin (Tongsong *et al.*, 1999 and Van Drriel *et al.*, 2002). In addition, few studies were recorded in rats and mice about warfarin (Feteih *et al.*, 1990 and Howe and Webster, 1990)and bromadiolone (Twigg and Kay, 1995) teratogenicity.

Marchini and Turillazzi (1978) mentioned that Racumin rodenticide treatment of lactating female albino rats induced 40% lethality on mothers while it caused 54% deaths in their offsprings. However, studies on Racumin teratogenicity and the mechanisms of its embryo/fetotoxicity need to be the primary research focus. Therefore, the present study gives an overview of the implications of in utero exposure to this coumarinic anticoagulant rodenticide for the developing fetus. In addition, new insights into the pathogenetic mechanisms of its embryo/fetotoxicity and teratogenicity are summarized.

MATERIAL AND METHODS

Three month old virgin female white rats, weighing 150-180 g were obtained from the National Institute of Ophthalmologic Research. Animals were kept for 2 weeks under our laboratory conditions and fed balanced food and tap water ad libitum. These females were examined periodically using the vaginal smear technique, to ensure that they were always in regular estrous. Each female in estrous was paired with a fertile male in a separate cage overnight. The presence of sperms in vaginal smear performed next morning was considered as zero day of pregnancy.

Pregnant females were randomly divided into 3 equal groups of 10 rats each. The first group kept without any treatment and served as control. The second and third groups were given 1.65 or 0.83 mg/kg b.wt. (1/10 and 1/20 LD_{50}, respectively, Tomlin, 1994) of Coumatetralyl, (Racumin: Bayer AG, Germany) respectively, daily from day 6 to day 15 of gestation (organogenesis period).

Teratological examination:

Treated and control pregnant rats were kept under observation for gross appearance and behavior till the 20th day of gestation when they sacrificed and teratological investigation was conducted according to Manson *et al.* (1982). Immediately after killing, the abdominal wall of the mothers was opened and the number of uterine implants and the number of resorptions were determined as well as the number of dead and live fetuses. The living and dead fetuses were distinguished immediately by the appearance of a moving reflex after touching the fetus in the unopened uterus of the mother with a pair of tweezers. The fetuses were numbered beginning with the upper end of the left uterine horn and ending at the upper end of the right one.

Individual fetal and placental weights were recorded, and gross examination for external fetal malformation was made macroscopically, Post- implantation loss was calculated. One third of the fetuses/litter were fixed in Bouin's solution for examining the visceral abnormalities using the free - hand razor blade sectioning method. The remaining two thirds of the fetuses from each group were fixed in 95% ethanol, eviscerated then cleared by 2 % potassium hydroxide and stained with alizarin red S – stain solution for examining the skeletal deformities.

Statistical analysis: Data are presented as mean ± standard error of the mean. The litter was regarded as the experimental unit of comparison for all analysis, except were otherwise noted. Treatment effects on fetal and placental weight and external morphological examination were determined by one way ANOVA. The Chi square test was used for the comparison of the different gross, visceral and skeletal anomalies between the groups. (Snedecor and Cochran, 1980).

RESULTS

No notable changes in behavior or clinical signs were observed in control or in treated dams at the two tested doses. No mortality was observed during the experimental period.

Table (1) summarizes the embryotoxic effects of the coumarinic rodenticide Racumin administered during the organogenesis period. The numbers of live fetuses per dam and the fetal body weight were significantly reduced in the treated group at 1.65 mg/kg b.wt. when compared to the other treated group at 0.83 mg/kg b.wt. and control one. The resorption (Fig. 1) and implantation sites (post implantation loss) (Fig. 2), dead fetuses number per dam and placental weights were significantly increased in both treated groups compared with the control group.

Gross examination of the fetuses from Racumin - treated groups Table (2) revealed significant incidences of stunted growth (Fig. 3), micrognathia, generalized subcutaneous edema and Subcutaneous hemorrhages (Fig. 4). The incidence of stunted growth was significantly higher in the treated group at 1.65 mg/kg b.wt. compared to the other treated and control groups.

The incidence of visceral anomalies in fetuses of control and treated groups was demonstrated in Table (3). Racumin significantly increased the total fetuses with visceral anomalies compared to the control. The visceral anomalies recorded in the treated groups were in the form of dilated brain lateral ventricles (hydrocephaly) (Fig. 5), dilated nares (Fig. 6), olfactory pulp hypoplasia, anophthalmia (Fig. 7), heart and lung hypoplasia, intrathoracic hemorrhages (Fig. 8), dilated renal pelvis (Fig. 9) and hydroureter.

There were significant skeletal anomalies observed in fetuses of Racumin treated groups (Table 4). They were; incomplete ossification of frontal, parietal, interparietal bones and sternebrae. In addition to large opened fontanels, deformed ribs, absence of metatarsal bones,

sacral and coccygeal vertebrae (Fig. 10). Also, there was a significant increase in the incidence of reduced sternebrae number (Fig. 11) and absence of metacarpal bones.The incidences of the recorded anomalies in the treated groups were dose – dependent.

Table (1): Racumin – induced embryo - toxicity in rats treated orally during the organogenesis period.

Parameters	Groups		
	Control	Racumin (1.65mg/kg b.wt.)	Racumin (0. 83 mg/kg b.wt.)
No. of pregnant dams	10	10	10
Total No. of implantations	110	108	109
Total No. of implants/dam	11.02 ± 0.25	10.65 ± 0.65	10.85 ± 0.40
No. of resorptions/dam	0.00	2.84 ± 0.45 [a]	1.95 ± 0.41 [a]
No. of dead fetuses/dam	0.10 ± 0.08	1.85± 0.33 [a]	1.45± 0.24 [a]
No. of live fetuses/dam	10.80 ± 0.44	3.95 ± 0.55 [ab]	5.00 ± 0.21 [a]
Post implantation loss (%)	0.10 ± 0.09	1.90 ± 0.25 [a]	1.45 ± 0.23 [a]
Fetal body weight (g)	6.95 ± 0.90	2.15 ±1.02 [ab]	4.70 ±0.95 [a]
Placental weight (g)	0.65 ± 0.08	1.19 ± 0.03 [a]	1.16 ± 0.08 [a]

The values are presented as mean ± S. E.
(a): Significant difference between treated and control groups at P< 0.05.
(b): Significant difference between the two treated groups at P< 0.05.

Table (2): Incidences of gross abnormalities in the fetuses of Racumin – treated rats during the organogenesis period.

Parameters	Groups		
	Control	Racumin (1.65mg/kg b.wt.)	Racumin (0. 83 mg/kg b.wt.)
Total No. of fetuses examined	109	60	66
Stunted growth	0	75 [ab]	45.45 [a]
Generalized subcutaneous edema	0	41.66 [a]	40.91 [a]
Subcutaneous hemorrhages	0	58.33 [a]	56.06 [a]
Micrognathia	0	33.33 [a]	30.30 [a]

The values represent percentage of gross abnormalities in relation to total number of fetuses' examined.
(a): Significant difference between treated and control groups at P< 0.05.
(b): Significant difference between the two treated groups at P< 0.05.

Table (3): Incidences of visceral abnormalities in the fetuses of Racumin – treated rats during the organogenesis period.

Parameters	Groups		
	Control	Racumin (1.65mg/kg b.wt.)	Racumin (0. 83 mg/kg b.wt.)
Total No. of fetuses examined	37	20	23
No. of fetuses with visceral abnormalities	1	14	13
Hydrocephaly	0	60 [ab]	43.47 [a]
Dilated nares	2.70	55 [ab]	39.13 [a]
Olfactory pulp hypoplasia	0	50 [ab]	34.78 [a]
Anophthalmia	0	65 [ab]	52.17 [a]
Heart hypertrophy	0	60 [ab]	47.83 [a]
Lung hypoplasia	0	65 [ab]	52.17 [a]
Intrathoracic hemorrhages	0	70 [a]	69.56 [a]
Dilated renal pelvis	2.70	55 [ab]	43.48 [a]
Hydroureter	0	50 [a]	47.83 [a]

The values represent percentage of visceral abnormalities in relation to total number of fetuses' examined.
(a): Significant difference between treated and control groups at P< 0.05.
(b): Significant difference between the two treated groups at P< 0.05.

Table (4): Incidences of skeletal abnormalities in the fetuses of Racumin – treated rats during the organogenesis period.

Parameters	Groups		
	Control	Racumin (1.65mg/kg b.wt.)	Racumin (0. 83 mg/kg b.wt.)
Total No. of fetuses examined	72	40	43
No. of fetuses with skeletal abnormalities	4	34	32
Incomplete ossification of skull	0	60 [ab]	46.51 [a]
Large opened fontanels	1.39	52.50 [ab]	44.19 [a]
Incomplete ossification of sternebrae	0	80 [ab]	67.44 [a]
Deformed ribs	0	50 [a]	48.84 [a]
Absence of metacarpal bones	0	75 [a]	65.12 [a]
Absence of metatarsal bones	0	72.50 [ab]	62.79 [a]
Incomplete ossification of caudal bones	2.77	85 [ab]	72.09 [a]

The values represent percentage of skeletal abnormalities in relation to total number of fetuses' examined.
(a): Significant difference between treated and control groups at P< 0.05.
(b): Significant difference between the two treated groups at P< 0.05.

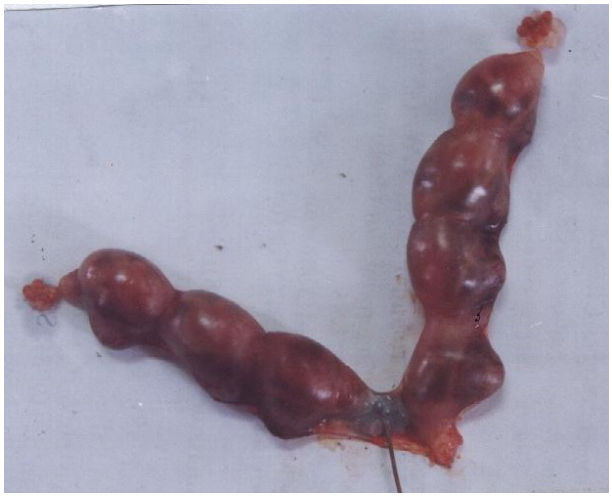

Fig. (1): Uterus of a pregnant rat treated orally with 0.83 mg Racumin/kg b.wt. during the organogenesis period showing a resorption site in the left horn.

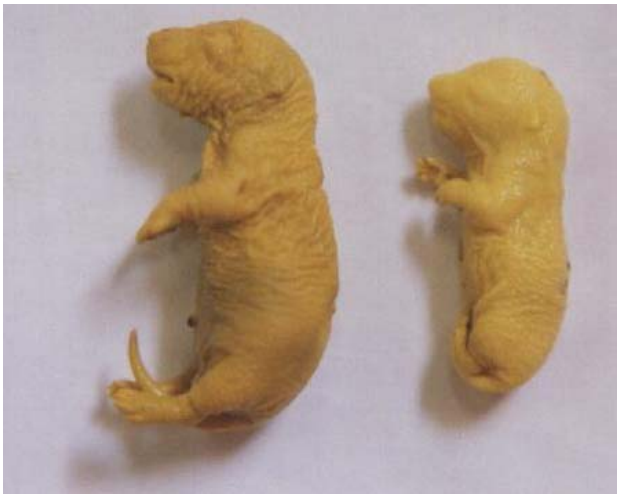

Fig. (3): Two rat fetuses, the left one is control, the right fetus obtained from a mother treated orally with 1.65mg Racumin/kg b.wt. during the organogenesis period showing stunted growth (dwarfism).

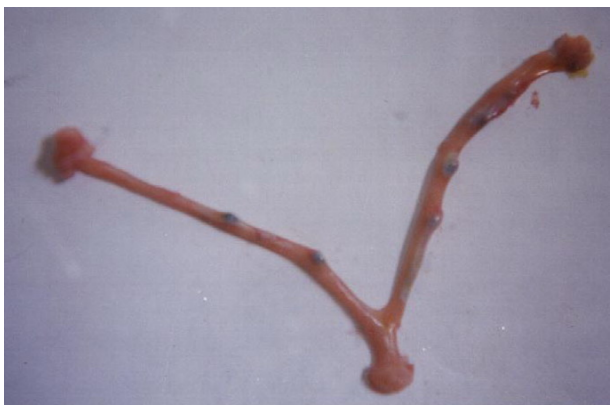

Fig. (2): Uterus of a pregnant rat treated orally with 1.65mg Racumin/kg b.wt. during the organogenesis period showing implantation sites (post implantation loss) in the right and left horns.

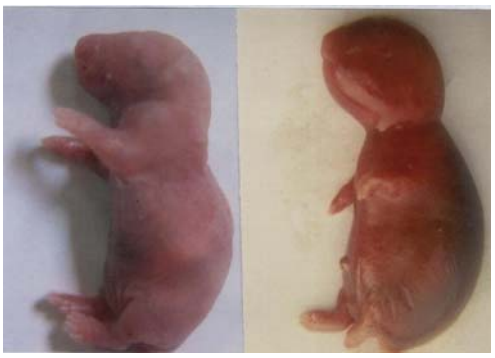

Fig. (4): Two rat fetuses, the left one is control, the right fetus obtained from a mother treated orally with 0.83 mg Racumin/kg b.wt. during the organogenesis period showing micrognathia, generalized subcutaneous edema and hemorrhages.

Fig. (5): A transverse section in the head of two rat fetuses, the left one is control, the right fetus obtained from a mother treated orally with 1.65mg Racumin/kg b.wt. during the organogenesis period showing dilated brain lateral ventricles(hydrocephaly).

Fig. (6): A transverse section in the head of two rat fetuses, the left one is control, the right fetus obtained from a mother treated orally with 0.83 mg Racumin/kg b.wt. during the organogenesis period showing dilated nares.

Fig. (7): A transverse section in the head of two rat fetuses, the left one is control, the right fetus obtained from a mother treated orally with 0.83 mg Racumin/kg b.wt. during the organogenesis period showing olfactory pulp hypoplasia and anophthalmia.

Fig. (8): A transverse section in the chest of two rat fetuses, the left one is control, the right fetus obtained from a mother treated orally with 1.65mg Racumin/kg b.wt. during the organogenesis period showing heart and lung hypoplasia and intrathoracic hemorrhages.

Fig. (9): A transverse section in the pelvis of two rat fetuses, the left one is control, the right fetus obtained from a mother treated orally with 1.65mg Racumin/kg b.wt. during the organogenesis period showing dilated renal pelvis.

Fig. (10): Two rat fetuses, the left one is control, the right fetus obtained from a mother treated orally with 1.65mg Racumin/kg b.wt. during the organogenesis period showing large opened fontanel, incomplete ossification of skull, metatarsal bones, sacral and coccygeal vertebrae and wavy ribs.

Fig. (11): Two rat fetuses, the left one is control, the right fetus obtained from a mother treated orally with 1.65mg Racumin/kg b.wt. during the organogenesis period showing incomplete ossification of sternebrae

DISCUSSION

The present study demonstrated the embryo- toxicity and teratogenicity of the coumarinic anticoagulant rodenticide, Racumin in the progeny of rats treated orally at a dose level of 1.65 or 0.83 mg/kg b. wt. during the period of organogenesis. Generally, there was no mortality or clinical signs of toxicity in the exposed animals at the two dose levels. Exposure of rats to Racumin at the two tested doses retarded fetal development and induced embryo - toxic effects as evidenced by decreased fetal weight, reduced number of live fetuses per dam and higher incidence of post implantation losses. The number of resorptions and dead fetuses was increased in exposed rats. The placental weight was significantly increased in both treated groups compared with the control group. However, the fetal body weight and numbers of live fetuses per dam were significantly reduced in the treated group at 1.65 mg/kg b.wt. when compared to the other treated group at 0.83 mg/kg b.wt. and control one.

Similar results were previously recorded with other coumarines (warfarin) (Feteih *et al.*, 1990 and Howe and Webster, 1990) in rats after exposure during the organogenesis and fetal periods. In addition, Twigg and Kay (1995) confirmed the adverse effect of bromadiolone (another hydroxycoumarin) rodenticide on the breeding performance of house mice (Mus domesticus) at sub-lethal doses equating to between 20% and 70% of the acute LD_{50} per feed.

Marchini and Turillazzi (1978) mentioned that coumatetralyl (Racumin) rodenticide treatment of lactating female albino rats induced 40% lethality on mothers (using a maximum concentration of toxic substance) while it caused 54% deaths in their offsprings. Among the probable causes of death in offsprings, the authors suggested the hypothesis that interference in the mother-offsprings relationship is due to the influence of coumatetralyl on parental behavior.

Teratogenic interference in the period of organogenesis, when most major organs and body regions are being established, is usually related to (major) structural anomalies. The fetal period is characterized by histogenesis and functional maturation; the influence of a teratogen in this period may cause growth retardation or functional disturbances (Carlson, 1999).

Freude *et al.* (1991), Greer (1998), Abadi *et al.* (2002) and Ageno *et al.* (2004) confirmed that coumarin derivatives including warfarin pass the placental membrane and has the potential to cause bleeding in the fetus and teratogenicity when used in early pregnancy. Coumatetralyl might be accumulated in the fetuses due to excessive transfer from maternal blood through placenta to fetus. Therefore, the impaired fetal physiology in Coumatetralyl - treated group resulting in embryo – and fetotoxic effects might be due to coumatetralyl accumulation as also seen in warfarin and other coumarine derivatives (Howe and Webster, 1990 and Tsakiris, 2005). In addition, the placenta plays an important role for the developing fetuses as it provides nutrition and hormonal regulation and transverse metabolic waste products. Disturbances in the placental microcirculation and hemorrhages induced by this rodenticide as evidenced by the increased placental weight may alter placental function and impair embryonic and fetal development. Consequently, the placenta may be directly involved in many instances of early spontaneous abortion and, fetal death and intrauterine growth retardation (Faulk, 1981).

The earliest concepts of the pathogenesis in the congenital anomalies found after in utero exposure to coumarin derivatives were based on the main clinical effects, the prolongation of blood clotting time. It was suggested that deformities in the child were caused by micro hemorrhages and subsequent scaring and calcification (Becker *et al.*, 1975). In light of these results, the early embryonic deaths noticed in female rats exposed to Racumin rodenticide most probably resulted from micro hemorrhages and consequently modification of the uterine lining function before arrival of the embryo and/or micro hemorrhages and subsequent scaring and calcification after implantation.

Gross examination of the fetuses from Racumin treated group revealed significant incidences of stunted growth, micrognathia and generalized subcutaneous edema and hemorrhages. Moreover, Racumin significantly increased the total fetuses with visceral anomalies compared to the control. The visceral anomalies recorded in the treated

group were in the form of dilated brain lateral ventricles (hydrocephaly), dilated nares, olfactory pulp hypoplasia, anophthalmia, heart and lung hypoplasia, intrathoracic hemorrhage, dilated renal pelvis and hydroureter.

Racumin - induced skeletal anomalies observed in fetuses of treated group were in the form of incomplete ossification of frontal, parietal, interparietal bones and sternebrae. In addition to large opened fontanels, deformed ribs and absence of metacarpal bones. Also, there was a significant increase in the incidence of reduced sternebrae number, absence of metatarsal bones and coccygeal vertebrae. The incidence of these anomalies was dose – dependent.

In agreement with our findings, case reports describing congenital anomalies after in utero exposure to coumarines and other related compounds in human include bleeding complications (intracranial or general visceral bleeding), growth retardation, microcephaly, hydrocephaly, dysfunction thermoregulation, facial,and skeletal anomalies (hypoplasia of distal phalanges, cervical vertebrae and short fingers), the so called warfarin embryopathy, as well as CNS malformations were found (Tongsong *et al.*, 1999 and Van Drriel *et al.*, 2002). A minority of structural malformation of other organs, including eye, heart and urinary tract were described after in utero exposure during early pregnancy (Van Drriel *et al.*, 2002). Blickstein and Blickstein (2002); Chan *et al.* (2003); Pattacini *et al.* (2003) and Finkelstein *et al.* (2005) recorded stillbirths, neonatal deaths and congenital abnormalities of the delivered fetus (included nasal hypoplasia, ear fold atresia, bilobled lungs, coarctation of the aorta, ventricular septal defect, gastrochisis,optic atrophy, hydrocephalus,mental retardation, cardiac structural defect and radiographic skeletal stippling) in mothers receiving prophylactic anticoagulation therapy during pregnancy.

Pati and Helmbrecht (1994) mentioned that optic atrophy and dilatation of the cerebral ventricles associated with blindness, microcephaly, and mental retardation have been reported following second and third trimester exposure of humans to warfarin.

The results of animal studies brought new insights into the pathogenesis of fetal anomalies associated with prenatal coumarin exposure. Coumarin derivatives as vitamin K - antagonists inhibit the recycling of vitamin K in the cell. In bone, cartilage, and the developing CNS, vitamin K – dependent proteins have been identified (Prieto *et al.*, 1999). In addition, animal studies confirmed an effect of warfarin on the developing bone and CNS (Howe and Webster, 1992 and Sundaram *et al.*, 1996). Coumarins interfere with vitamin K recycling in the cell by inhibition of vitamin K epoxide reductase (VKOR), which leads to the depletion of hydroquinone (KIH2). KIH2 is cofactor for γ–glutamyl carboxylase, a vitamin K-dependent (VKD) enzyme which mediates posttranslational modification (carboxylation) of glutamyl (Gla) residues needed for biological activity of coagulation factors (II, VII, IX, and X), and its depletion results in the accumulation of undercarboxylated (inactive) intracellular precursors of several VKD proteins involved in the coagulation process. In this way, warfarin affects the catalytic rate of vitamin K dependent proteins involved in normal hemostasis (Furie, 2000). The observed generalized subcutaneous edema and hemorrhages in the obtained fetuses from Racumin treated female rats in our study may be attributed to the disturbed coagulation and clotting mechanisms induced by this coumarin rodenticide.

By inhibiting VKOR, however, warfarin affects the generation of biologically active VKD proteins required for biological processes other than hemostasis, including proteins involved in the regulation of bone growth and calcification (Price, 1988) and VKD proteins involved in mesangial and vascular smooth muscle cell growth (Nakano *et al.*, 1997 and Yanagita *et al.*, 1999). The effects of warfarin on bone Gla protein (BGP/osteocalcin) and matrix Gla protein (MGP) are responsible for developmental defects (warfarin embryopathy) when warfarin is administered during pregnancy (Price *et al.*, 1982) and bone mass loss in patients on long – term anticoagulant therapy (Resch *et al.*, 1991). Suppression of MGP in humans is a possible determinant in arterial calcification (Price *et al.*, 2000). Moreover, Feteih *et al.* (1990) observed a disruption in the columnar arrangement of the hypertrophic chondrocytes in cartilage of fetal rats after warfarin exposure. This was attributed to overmineralization or the result of a more fundamental disturbance of the chondrocytes.The recorded skeletal anomalies in the fetuses from coumatetralyl treated pregnant rats in our study may be due to a warfarin - like effect on bone development.

The effects of coumarins on the developing CNS may be indirect due to hemorrhages, or direct, through interference with normal development of the brain (Van Drriel *et al.*, 2002). In the CNS, vitamin K has been shown to stimulate the activity of at least 2 microsomal enzymes in the sphingolipid pathway (Sundaram and Lev 1990). The requirement of phosphorus in these experiments suggests that a phosphorylation step is involved (Sundaram and Lev, 1990). End products of this pathway are cerebrosides,sulfatides, gangliosides and sphingomyelin. Sphingolipids are important structural compounds of myelin, but they also serve as second messenger in intracellular signal transduction pathways (Tsaioun, 1999). Sundaram *et al.* (1996) showed that warfarin administration resulted in a significant reduction of sulfatides in mouse brain through inhibition of the activity of galactocerebroside sulfotransferase (GST).

Recently, a new vitamin K–dependent protein (Glu-protein) was discovered, Gas 6 (Manfioletti *et al.*, 1993). This growth factor is a ligand for a subfamily of the receptor tyrosine kinases (RTKs). RTKs are receptors with an extracellular domain, which bind the ligand and an intracellular tyrosine kinase that is important for signal transduction (Varnum *et al.*, 1995). RTKs were first described for their role in cell growth and proliferation, but are also known to function in cell migration, axonal path

finding, cell survival and neural type determination. Gas 6 and its receptor, Tyro 3, were found to be widely distributed throughout the CNS (Prieto *et al.*, 1999). In the brain of developing chick embryos, tyrosine phosphorylation was enhanced by vitamin K; wherease warfarin was shown to inhibit this phosphorylation cascade (Saxena *et al.*, 1997). Inhibition of regulatory growth factors like the vitamin K – dependent Gas 6 might cause disorganization of the CNS during development (Van Drriel *et al.*, 2002). Besides the CNS, Gas 6 has a wide tissue distribution, e.g., lung, intestine, heart and testis (Tsaioun, 1999). Therefore, it is conceivable that Gas 6 inhibition might have a negative influence on the embryogenesis of other organs. In light of these studies, defective brain embryogenesis due to exposure to Racumin during the organogenesis period may be the cause of the recorded dilatation in brain ventricles (hydrocephaly) in our results.

WHO (1995) classified warfarin and other hydroxycoumarins (coumachlor and coumatetralyl) as highly dangerous agents that induce effects on proteins not dependent on vitamin K (nonVKD proteins), thereby, affecting cell growth (Nakano *et al.*, 1997 and Yanagita *et al.*, 1999), signal transduction (Kater *et al.*, 2002) and inflammatory/immune system cell activity (Avanzi *et al.*, 1998 and Kataranovski *et al.*, 2003).

Another possible cause of developmental toxicity is the genotoxicity (Manson *et al.*, 1982). Coumarins genotoxicity has been demonstrated in Chinese hamster ovary cells exposed to coumarin (NTP, 1993). This may be another mechanism for the induced embryo/fetotoxicity and teratogenicity in our results.

It can be concluded that Racumin is an embryotoxic and teratogenic rodenticide when administered during the organogenesis period. This embryotoxicity can be produced even at low doses which may not manifest any alarming clinical signs of toxicity in exposed dams. Therefore, it is recommended that animals must be kept away from gaining access to either Racumin- containing rat baits or Racumin- poisoned rats specially during pregnancy.

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