Amendment Effect of Antioxidants of Barley and Oat against Teratogenicity Induced by Amitraz

Omina, I. Ali [1], Hanaa, M.R. Hegazy*[2] and Fatma,M.Fakhry [1]

[1] Toxicology Dept. ,Animal An. Health Res. Inst., Cairo, Egypt
[2] Toxicology Dept., Fac. of Vet. Med., Kafr El-Sheikh University, Kafr El-Sheikh, Egypt
*drhantox@yahoo.com

Abstract: The present study investigated the protective effect of phytonutrients and antioxidants in barley and oat added to diet on teratognic effects induced by amitraz® after maternal exposure during pregnancy. 40 pregnant albino rats were divided into 4 groups each of 10. one group was kept as control fed the balanced ration& administered distilled water. Three groups were administered amitraz® (50 mg/ kg b.w.) by gavage during organogenesis from days 6 through day15 of gestation, a group of them fed the balanced ratio and the other 2 groups fed the same diet supplemented with barley or oat (20%), 4 days prior of gestation and continued till the end of the experiment. All dams underwent a caesarean section on day 20 of gestation and their fetuses were examined for external, visceral and skeletal abnormalities. Amitraz induced maternal toxicity manifested as lower body weight gain, developmental toxicity included fetal death, a decrease in fetal body weight and length, as well as increased incidence of fetal external, visceral & skeletal anomalies. These findings were prevented or in lower incidence in groups provided with barley or oat. The obtained data were reviewed and discussed. Conclusively, barley and oat supplement have protective and positive modulation response due to their phytonutrients and antioxidants against congenital anomalies induced by amitraz®. In recommendation, the incorporation of cereal grains such as barley and oat in the food products are chemopreventive agents for adverse effects of xenobiotics.
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1. Introduction:

Amitraz is a triazapentadiene compound, a member of the amidine chemical family. It is an insecticide and acaricide used in agriculture and veterinary medicine. It is used to control ticks, mites, lice and other animal pests (Institoris *et al.*, 2007). Amitraz poisoning can occur through oral or dermal routs and potentially by inhalation. Poisoning cases were reported in both human and animal (Proudfoot, 2003). The most common adverse effects associated with amitraz are central nervous system alterations (Yilmaz and Yildizdas, 2003; Kim *et al.*, 2007). There are limited data on reproductive and developmental toxicity due to amitraz. Landmarks alterations due to amitraz exposure in rats were recoded by Palermo – Neto *et al*.(1994) and (1997) prenataly and posnataly respectevly . Osano *et al.* (2002) reported that exposing the frog to amitraz resulted in an increase in the incidence of edema, pigment loss and encephalomegaly. Al–Thani *et al.* (2003) recorded a decrease in fertility index, sperm production and postimplantation loss in male mice exposed to amitraz (40 ppm) in drinking water. Kim *et al.* (2007) studied developmental toxicity in rats given amitraz during gestation.

Phytonutrients are abundant in whole grains such as barley & oat which protect against cancer and heart diseases. Dietary modulation immune functions by barley or oat β– glucans is useful to immune system & increase resistance against pathogens (Voiman *et al*, 2007), lower high cholesterol, LDL & triglycerides (Ames & Rhymer, 2008; Nancy and Camille, 2008). Barley and oat are dietary sources of water soluble, fat soluble & insoluble antioxidants include vit. E, tocotrienals; phenolic & phytic acids, flavons, flovonoids, selenium as well as good sources of amino and fatty acids, sugar, carbohydrate, vitamins, β-glucans, minerals Ca, P, Na, K, F, Cu, Zn, Mn, & Mg (Erkkila *et al.*, 2005; Djousse & Gaziano, 2007; Kurtz & Wallo, 2007, Lee- Manion *et al.*, 2009 and Gallagher *et al.*, 2010). Therefore, the aim of this work was to evaluate the amendment effect of barley & oat supplement against teratogenicity induced by amitraz during pregnancy at the period of organogenesis in albino eats.

2. Material and Methods

Amitraz [N, N – (methylimino) dimethylidyne], a formamidine insecticide and acaricide was obtained from ADWIA company Egypt, Barley (Hordeum Vulgare L) and oat (Avena sativa L) were used in this work.

Experimental animals:

40 females & 20 males adult albino rats of Wistar strain were used in this study.

Experimental Design:

Rats were divided into 4 groups each of 10. one group was kept as control, administered sterilized distilled water & fed the balanced ration. The other Three groups administered by gavage, amitraz (50 mg / kg b.w.) from days 6 through day 15 day of gestation and fed the same diet, except 2 groups supplemented with barley or oat as 20% of diet, 4 days prior gestation and continued during gestation till the end of the experiment.

Teratogenicity study:

Experimental rats were mated overnight with control males and the next morning, the presence of sperms in vaginal smear was considered to be 0 day of pregnancy. Initial and final maternal body weight was measured. At the scheduled termination, day 20 of gestation, all dams were euthanized by ether inhalation and caesarian sections were performed. The ovaries and uteri from each female were removed and examined for the number of corpora lutea, status of all the implantation sites (ie, live & dead fetuses, early and late resorptins and total implantations). The uteri with no evidence of implantation were stained with a 2% sodium hydroxide solution to identity the presence of early resorptin sites (Yamada *et al.*, 1985). If no stained implantation sites were present, the rat was considered not pregnant. Resorption was classified as "early" when only a resorption site resembling a dark brown clot and with no embryonic tissue was visible and it was classified as "late" when both the placental and embryonic tissues were visible at the post – mortem examination. All live fetuses were removed, dried on absorbent paper, individually weighed, sexed, measured the crown- rump length and examined for any morphological abnormalities including a cleft palate. Alternative fetuses were selected for either a skeletal or visceral examination. Half of live fetuses from each litter was fixed in ethanol, cleared with KOH and stained with Alizerin red S and examined for skeletal malformations (Staples & Schnell, 1964). The other half was preserved in Bouin`s solution and examined for internal soft tissue changes using a free hand razor sectioning technique and examined under a dissecting microscope (Wilson 1972; Nishimura, 1974).

Statistical analysis: of quantitative data were subjected to "t" test, while the incidence of fetal anomalies using litters was analysed by the Fisher`s exact probability test and the Mann – Whitney U test. The significance level for each statistical comparison was reported at $P < 0.05$ using software SPSS (2006).

3. Results

Table 1, summarizes the maternal and fetal findings for dams treated with amitraz. The number of implantations, placental weight and the gender ratio of live fetuses were similar among the experimental groups. However, the number of fetal deaths including resorptions and dead fetuses was significantly higher, whereas fetal body weight and length were lower in amitraz – treated group than the control and the supplemented groups with barley or oat. Table 2 shows the fetal external abnormalities with a significantly higher incidence in amitraz – treated group in comparison with the supplemented and control groups.

Fetal visceral and skeletal anomalies were recorded in Tables (3 & 4), respectively. Malformations were severe with high incidence in the group given amitraz only, versus the supplemented groups with barley or oat.

Table(1):Effect of barley (B)or oat(O)supplement on fetal maternal and fetal values from dam given amitraz(A)50/kg by gavage through 6-15days of gestation.

Parameter \ Group	Control	A	A±B	A±O
Maternal weight gain.	63.6 ±0.5	*31.3±.055	*54.5±1.33	0.78±*56.1
No. Of corpora lutea.	12.2 ±0.2	12.25±0.4	12.4±0.2	12.3±0.3
No. of implantation sites	11.9±0.2	11.9±0.4	12.0±0.3	11.9±0.2
Resorption :Early	0.2±0.01	*1.1±0.01	0.5±0.04	0.5±0.02
.Late	0	*4.4±0.03	1.6±0.05	1.4±0.03
Live fetuses.	11.7±0.5	*6.2±0.12	9.9±0.4	10.0±0.22
Dead fetuses.	0	*0.2±0.01	0	0
Fetal weight(g)	4.0±0.22	*2.2±0.28	3.5±0.3	3.3±0.5
Fetal length(cm)	3.7±0.3	*2.0±0.1	3.2±0.33	3.0±0.5
Sex ratio(M/F)	1.2±0.02	1.57±00.3	1.35	1.26
Placental weight(g)	0.5±0.04	0.7±0.02	0.62±0.02	0.64±0.05

*Significantly different form controls using" t"test and *,Mann-Whitney U test at P<0.05

Table(2): Effect of barley (B)or oat(O)supplement on fetal external abnormalities from dam given amitraz(A)50/kg by gavage through 6-15days of gestation.

Group / Parameter	Control	A	A±B	A±O
Fetuses examined.	116	69	98	100
Cleft palate	0	15(*24.2)	0	2*(2)
Hydrocephaly.	0	13(*21)	0	0
Hematoma.	0	15(*24.2)	5(*5.1)	5*(5)
Micromelia.	0	20(*32.25)	7(*7.4)	5*(5)
Syndactyly.	0	12(*19.4)	0	3*(3)
Tail:short or stunt .	0	21(*33.87)	10(*10.2)	8*(8)
Anal atrasia.	0	11(*17.74)	3(*3.1)	3*(3)
Improforated anus.	0	11(*17.74)	5(*5.1)	3*(3)
No genital papillae.	o	10(*16.13)	0	0

Values indicate the No. malformed fetuses (average %of malformed fetuses per liter)
Significantly different form controls by Fisher's exact test at P<0.05.*

Table(3):Effect of barley (B)or oat(O)supplement on fetal visceral anomalies from dam given amitraz(A)50/kg by gavage through 6-15days of gestation.

Group / Parameter	Control	A	A±B	O±A
Fetuses examined	58	31	49	50
Stenosis of olfactory bulb .	0	11(*35.5)	2(*4.1)	2(*4)
Oblique nasal septum .	0	7(*22.6)	2(*4.1)	3(*6)
Anophthalmia and/or microphthalmia.	0	23(*74.2)	5(*10.2)	3(*6)
Internal hyprocephaly.	0	6(*19.4)	0	0
Brain hydrotrophy.	0	11(*35.5)	3(*6.1)	3(*6)
Dilatation of lateral and/or 3rd ventricle.	0	18(*58.1)	6(*12.2)	5(*10)
Cranial hemorrhage.	0	5(*16.1)	1(*1.1)	1(*2)
Heart hypertrophy.	0	15(*48.4)	2(*4.1)	5(*10)
Ventricular septal defects.	0	8(*25.81)	2(*4.1)	2(*4)
Small lung.	0	11(*35.5)	6(*12.2)	5(*10)
Small spleen.	2(3.51)	7(*22.6)	7(*14.2)	7(*14)
Large stomach.	0	7(*22.6)	3(*1.6)	4(*8)
Hepatomegaly.	0	12(*38.7)	2(*4.1)	0
Enlarged adrenals.	0	7(*22.6)	0	0
Renal agenesis and/or hypertrophy.	0	18(*58.06)	3(*66.1)	3(*6)
Misshapen and/or malpositioned kidney.	1(1.7)	7(*19.4)	2(*4.1)	3(*6)
Dilated renal pelvis.	0	15(*48.4)	4(*8.2)	3(*6)
Testis agenesis and/or hypotrophy.	0	15(*48.4)	3(*6.1)	3(*6)
Undescended testis.	0	7(*22.6)	2(*4.1)	2(*4)

Values indicate the No. malformed fetuses (average %of malformed fetuses per liter)
*Significantly different from controls by Fisher's exact test at P<0.05.

Table(4): Effect of barley (B)or oat(O)supplement on fetal skeletal malformations from dam given amitraz(A)50/kg by gavage through 6-15days of gestation.

Group / Parameter	Control	A	A±B	A±O
Fetuses examined.	58	31	49	50
Skull:cranioschisis,wide cranial suture.	0	12(*38.71)	0	0
Incomplete ossification of parietal,interparietal and occipital bones.	0	23(*74.2)	5(*10.2)	7(*14)
Vertebrae:Fused cervical.	0	10(*32.3)	0	0
Lumbar Centrum :misaligned,.	0	22(*71)	7(*14.3)	9(*18)
Unossified,Bipartite	0	11(*35.5)	0	0
Wide intercostal space.	0	9(*62.9)	3(*6.1)	3(*6)
Sacrocaudal vertebra (fused,and/orincomplete ossification)	0	21(*67.74)	7(*14.3)	9(*18)
Sternebrae:Malaligned.	2 (3.5)	7(*22.6)	5(*10.2)	6(*12)
. Misshapen	0	15(*48.39)	4(*8.2)	3(*16)
Bipartite.	0	16(*51.61)	4(*8.2)	2(*14)
. Surplus	0	2(*6.4)	0	0
Scrambled.	0	7*(22.6)	0	0
Unassified .	0	11(*35.48)	3(*6.1)	3(*16)
Ribs:Fused	0	7(*22.6)	0	0
Wavy	0	12(*38.71)	7(*14.3)	9(*18)
Rudimentary	0	6(*19.35)	0	0
. Shortened	0	18(*58.07)	9(*18.4)	11(*22)
Limbs:Fused/incomplete ossification of metacarpal and/or bones.	0	16(*51.61)	6(*12.2)	8(*16)
Unassified Centrum of phalanges.	0	12(*15.48)	0	0

Values indicate the No. malformed fetuses (average %of malformed per liter)

*Significantly different from controls by Fisher's exact test at P<0.05.

4. Discussion

Amitraz is a pharmaceutical veterinary and agricultural product which is used worldwide under numerous generic names as an acaricide and insecticide (Yilmaz & Yildizdas, 2003). This study investigated the potential chemoprotective effects of phytonutrients and antioxidants of barley and oat against teratogenicity induced by amitraz. The results show that, groups of pregnant albino rats supplemented with barley or oat, completely prevent severe terata & provoked positive protective response towards the incidence of congenital malformations in fetuses obtained from dams given amitraz (50 mg / ky) by gavage during pregnancy at the period of organogenesis on days 6 through 15 days. Amitraz–treated group without supplement, showed a decrease in maternal body weight gain, which may be attributed to post implantation loss and/or the decreased fetal body weight. Amitraz induced the embryo–fetal development toxicity, which included increased fetal death, post impartation loss, decreased fetal body weight and length as well as increased fetal external, internal and skeletal anomalies. The reduction in fetal body weight is classified as an indicator of intrauterine growth retardation (Kim *et al.*, 2004). The accurrence of a delay in ossification in several skeletal districts coincided with the decreased fetal body weight.

This is consistent with the results of Osano *et al.*, (2002) in frog exposed to amitraz and resulted in high incidence of encephalomegaly. Kim *et al.*, (2007) showed that a 19 – day oral repeated dose of amitraz during pregnancy is embryotoxic & teratogenic in rats at the maternally toxic dose (10 – 30 mg / kg).

Amitraz and its degradant 2, 4 – dimethylaniline is genotoxic, teratogemic and carcinogenic (Brimecombe *et al.*, 2006). Amitraz blockes monoamine oxidase activity in liver and brain (Bonsall & Turnbull, 1983); increases the levels of noradrenalin & dopamine in brain (Florio *et al.*, 1993); induces CNS alterations (Yilmaz and

Yildizdas, 2003; Kim *et al.*, 2007). During development the CNS can be especially susceptible to the toxic xenobioties due to maternal exposure during gestation and/or lactation, causing developmental neurotoxicity and/or behavioral abnormalities in the offspring that may persist throughout the lifetime (Francis *et al.*, 1990).

In this work, it is worthy to mention that both barley and oat supplement are in the same order of magnitude in preventing or inhibiting the incidence of congenital malformations of fetuses obtained from dams exposed to amitraz during period of organogenesis of pregnancy.

Tocotrienols highly enriched in barley and oat have antioxidant and potent anticancer properties (Colombo *et al*., 2009; Sun *et al*, 2009). Phenolic acids in barley and oat possess high antioxidant antiradical, antiproliferative potentials, oxygen and hydroxyl radical scavenging capacity and potency in prevention of lipid oxidation (Madhujlth & Shahidi, 2007; Gallagher *et al.*, 2010) as well as β-glucons, which increase immune defence by activating complement system that improve regulation against oxidative damage and genotoxicity authorizing a health claim (Akramiene *et al*., 2007; Voiman *et al*., 2007).

Barley has antioxidant activity via total phenols, flavons and flavonoids as protective compounds (Atrooz, 2009; Ferreres *et al.*, 2009; perez-lopez *et al.*, 2010) Barley seeds contain 3, 4–Dihydroxy–benzaldehyde, which inhibits oxidative DNA damage and apoptosis via its antioxidant activity by blocking H2O2 (Kanauchi et al.,2008;Jeong *et al.*, 2009). Anthocyanin compostion and oxygen radical scavenging capacity of barley were reported by Bellido and Beta (2009); as well as melatonin (N–acetyl–5–methoxy tryptamine) (Hernandez – Ruiz and Amao, 2008); and Lunasin, cancer preventive peptide (Hernandez–Ledesma and de Lumen, 2008). Oat contains a number of antioxidants such as tocols, ferulic acid and phenolics such as avenanthramides, alpha-linolenic acids, omega-3, omega-6 fatty acids (Moreau *et al*., 2008; Gallagher *et al*., 2010). B-glucans and fat content in oat are higher than most other grains. The presence of different types of phenols in oat confers antioxidant and antinflammatory (Kurtz & Wallo, 2007). Avenanthramides, the unique alkaloid phenols, exert antioxidant and antigenotoxic activities (Chen *et al*., 2007; Lee – Manion *et al.*, 2009; Gallagher *et al.*, 2010), increase the stability of the genetic protein P53, which is a critical factor in the reduction of cell mutation as regulates tumor necrosis factor (Nie *et al.*, 2006; Guo *et al*., 2008; Wise *et al*., 2008).

Conclusively, the current study indicates that the inclusion of whole barley or oat into the daily diet render health benefits as well as protection against teratognicity induced by amitraz. It is recommended that the incorporation of cereal grains such as barley and oat in the food products improves not only the nutrition, but also a therapy against genotoxicity, teratogenicty and carcinogenicity induced by xenobiotics.

Corresponding author:
Hanaa, M.R. Hegazy
Toxicology Dept., Fac. of Vet. Med., Kafr El-Sheikh University, Kafr El-Sheikh, Egypt
drhantox@yahoo.com

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