Pregnancy and fetal correlations of cypermethrin in mice

(Mus musculus)

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ABSTRACT

In the present study Cypermethrin, [(R, S) \( \alpha \)-cyano-3-phenoxybenzyl (1R, S)-cistrans-3-(2, 2-dimethylcyclopropane carboxylate) was tested for its capacity to induce pre and peri and pan-gestational losses and teratological changes in albino laboratory mice (Mus musculus). After careful estimation of LD\(_{50}\) for pregnant mice (64mg/kg body weight) 5, 10 and 20% (i.e. 3.2, 6.4 and 12.8mg/kg BW of the dams) of LD\(_{50}\) were chosen as experimental doses. A group of 20 Dams was maintained as control. Experimental doses were applied on GD6 and GD6, 9 and 12 (called single and triple exposures respectively) to a faction of 20 Dams each. Fetuses were exteriorized on GD18 to estimate pre, peri and pan-implantational losses. Fetuses were subjected to study for density/ litter/ faction, Crown Rimp Length (CRL) and Head circumference.

Results indicate a dose and exposure dependent increase in pre, peri and pan-gestational losses. Maximum fetal density was recorded in 3.2mg/kg single exposure sub-group. While in single exposure factions of the remaining two groups it showed dose and exposure related decline. A secondary decline in fetal density was noted in all groups at triple exposure. A dose dependent increased frequency of feto-morphic defects including, microcephaly, hydrocephaly, un-detached pinnae, epinate ears, skewed neck, meromelia, extradactyly, drooping wrist, round back, hemoregia, torted hid limbs, forked paws, flipper limbs and kinky tail were observed. Fetal head circumference and CRL also showed a dose dependent decline.

Results indicate that cypermethrin is a potent developmental toxicant. It brings about various morphological abnormalities along with significant changes in morphometric data.

Key Words: Cypermethrin, Pan-gestational losses, Fetal derangements

INTRODUCTION

Fetal development is a dynamic process that includes changes in morphology, anatomy, physiology, biochemistry and general growth (Roger & Kevlock, 2001). Exposure to various environmental chemicals (especially pesticides) during developmental period is liable to give rise to congenial defects (Akhtar, et al. 2006). By now there exist a vast number of evidences that indicate the susceptibility of developing embryos to environmental toxicants especially insecticides (Gupta, 1990; Roy, et al. 1998; Tian, et al. 2005; Slotkin, et al. 2006). The environmental agents interfere with the developmental processes thus derailing them from giving their proper end results (Uggini et al. 2010).

Organophosphates and pyrethroids are the two most commonly used groups of pesticides that are, unfortunately, also known to influence embryonic
development in non-target animals (Ahmad & Asmatullah, 2007, Uggini, et al. 2010). Cypermethrin [(R, S)-\(\text{\(\alpha\)}\)-cyano-3-phenoxybenzyl (1R, S)-cistrans-3-(2, 2-dimethylcyclcopropane carboxylate] is a type II pyrithroid insecticide that is currently being used in agriculture, work places and domestic sectors in developing countries in Asia (Abhilash & Singh, 2009). It is one of the most frequently used insecticides in Pakistan (Ahmad, et al. 2009).

Cypermethrin is a lipophilic substance that forms long term associations with integral membranous proteins (Michelangeli et al., 1990), particularly the ATP dependent ion channels (Prashanth & David, 2010). In this way it enhances the permeability of Na\(^+\) channels (Kumar, et al. 2009) simultaneously altering the function of transient K\(^+\) channels, thus inducing repetitive impulses in neurons (Smith & Soderlund, 2001; Gowland et al., 2002) causing loss of coordination, muscular tremor, and convulsions (Desi et al., 1986). Cypermethrin has been found to induce DNA damage (Patel et al., 2006) and chromosomal aberrations (Kocaman & Topaktas, 2009), disturb the activities of sex steroids (Waters, et al., 2001; Chen et al., 2002; McCarthy et al., 2006) and cause hormonal disruptions (Xu et al., 2008). Evidences indicate that cypermethrin exposure inflicts oxidative stress leading to DNA damage and apoptosis (Jin et al., 2010).

Along with its systemic and cellular toxicological implications (Muthuviveganandavel et al., 2008; Ahmad et al., 2009; Khan et al., 2009, Sharaf et al., 2010); there exist convincing number of evidences that exposure to cypermethrin in males have led to changes in reproductive organs and sperms leading to a decline in the reproductive success (Elbetieha et al., 2001; Kumar et al., 2004; Ahmad et al., 2009; Rodriguez et al., 2009; Wang et al., 2009, 2010; Al-Hamdani & Yajurvedi, 2010).

According to Shukla & Taneja (2002) cypermethrin brings about germ cell mutations and chromosomal aberrations that lead to death and resorptions of embryos in mouse. There are relatively few studies on cypermethrin dealing directly with gestational exposure leading to various damages to the developing fetuses (Gupta, 1990; Ullah et al., 2006, Farag et al., 2007, Uggini et al., 2010). Farag, et al. (2007) have shown significant delay in down appearance and eye opening in fetuses along with delayed development and detachment of pinna at 10mg/kg cypermethrin per-gestational exposure for 4 weeks /5days in a week in virgin mice. Ullah, et al. (2006) have found pre and post-implantation losses following (ip) cypermethrin exposure at 25, 50, and 75 mg/kg BW in rabbit on post-mating day5, 10, 15, and 20. In a recent study Uggini, et al. (2010) have reported alterations in fetal growth especially in the axial and appendicular skeletal development following cypermethrin exposure, given in combination with chlorpyrifos (5 and 50% respectively) in chick eggs, on incubation day0 at 0.5\(\text{L}\) / egg.

The above cited literature indicates embryonic disruptions. Cypermethrin exposure at high dose levels may be toxic to the adults as well. Thus the present study was designed to explore all kinds of fetomorphomorphic derangements at motherly safe dose exposures.
MATERIALS AND METHODS

This study was conducted on 140 pregnant Swiss Webster laboratory mice, ageing between 3 to 4 months weighing 30±3 grams. They were assigned randomly to various groups (Fig 1). The Dams were kept under 12-12-hrs dark light cycles, in 12” x 12” x 18” metal gauzed steel framed cages. Fine cuttings of 40g brand new infection free white paper were provided for bedding. The bedding was replaced twice a week. Ambient temperature 23 ± 2°C and humidity 35-40% were maintained. Pallets of specially prepared rodent food and water were provided ad libitum. Appropriate dilutions (using corn oil) of commercial formulation of Cypermethrin (10EC) imported and marketed by Warble private limited (Batch: 33080712-A) were used for experimental exposure.

As no information about LD50 values for pregnant mice available in literature, it was decided to estimate it carefully. A wide range of exposure doses (i.e. 25, 50, 75, 100 and 125mg/kg) was selected and tested for the estimation of the LD50 values for the pregnant females. To determine mortality (number of animals died within 48hours of exposure) at these exposure doses; each dose was tested on a group of 10 pregnant females. Inline with the probit analysis procedure enumerate by Finney, (1971) the data obtained was subjected to "regression curve analysis" for statistical estimation of required LD50 values (Fig 2).

After careful estimation of LD50 for pregnant mice 20, 10 and 5%of this reference dose (i.e. 12.8, 6.4 and 3.2mg/kg BW respectively) were selected as experimental doses. Each experimental dose was applied in two ways to a faction of 20 animals that is as single (on GD6) and multiple (on GD6, 9 and 12). A group of 20 animals was also maintained as vehicle exposed control (Fig 1). All doses to the experimental animals were applied by gavage.

All females were etherized and weighed just before the fetal recovery on GD18. The fetuses were exteriorized by means of a mid ventral incision in each case. Both horns of the uterus were exposed and finally removed intact, in each case, and the number of implantations were counted in situ. Both ovaries were also removed from each animal to count the corpora lutia under a dissecting research binocular microscope at 20X. Finally with help of fine scissors the graved uteri were opened and all fetuses (live, dead and resorbed) were recovered. Data obtained, at this stage, was represented in the result section on the following bases.

1. Total number of implantation sites/ animal/ group
2. Total number of corpus lutia in both ovaries/ animal/ group
3. Number of live fetuses/ litter/ group
4. Number of dead + resorbed fetuses/ litter/ group
5. Pre, peri and pan-gestational losses/ litter/ group
6. Pregnancy indices in each group

The total number of the corpora lutia and implants were counted in situ from the ovaries and the uterus of each experimental animal as explained above. The pregnancy index was calculated by means of the following equations as reported by Kar, et al. (1984) and Shukla & Taneja (2002).
Pregnancy Index (%) = 100 – % Co-gestational losses

Where as

\[
% \text{ Co-gestational losses} = \frac{\text{Total No of Corpora lutia / Female} - \text{Total No of implants /female} \times 100}{\text{Total No of corpora lutia / female}}
\]

The morphological study involved a careful observation of each fetus under a binocular dissecting research microscope (Labomed CXM2) at 10 and 20x. Photomicrographs of the selected fetuses were obtained with the help of digital camera (Kyocera MR410) fitted on a mechanically adjustable stage in super macro-mode.

The morphometric studies involved statistical analyses of the data regarding fetal weight, Crown-rump (CR) and head circumference. To obtain mean fetal weight/ litter, all fetuses in a litter were weighed together on a 0.001mg precision digital balance (Sartorius TF 214S) and the value obtained was divided by the total number of fetuses in that litter. On the other hand all fetuses in each litter were subjected individually for the measurements of CR length with the help of a vernier caliper. Similarly the occipito-frontal length and bi-parietal distance for each fetus were obtained to calculate fetal head circumference with the help of the following formula used for the calculation of circumference of an ellipsoid.

\[
P = 2\pi \sqrt{\frac{a^2 + b^2}{2}}
\]

Where \( a = \frac{\text{occipito-frontal length}}{2} \) and \( b = \frac{\text{bi-parietal distance}}{2} \)

Fetal density can provide a direct indication of the effect of gestational cypermethrin exposure on the extant of fetal bone ossification. In order to calculate the fetal density/ litter; mean/ litter values of fetal weight and volume were obtained with the help of a digital balance (Sartorius TF 214S) and measuring cylinder of 10ml, respectively. Mean/ litter values of fetal density were calculated by using the following formula.

\[
\text{Mean fetal Density/ litter} = \frac{\text{Mean fetal weight in a litter}}{\text{Mean fetal volume in a litter}}
\]
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RESULTS

LD$_{50}$

Various doses of Cypermethrin (25, 50, 75, 100 and 125mg/kg BW) were given to a group of 10 pregnant mice each. Mortality at each of these doses was plotted against a linear regression trend line; for the statistical estimation of the lethal dose for 50% mortality. The calculated LD$_{50}$ of cypermethrin for the pregnant mice was 64mg/kg BW (Fig. 2).

Pregnancy index

Mean litter size (live fetuses/ litter) and pregnancy index followed the dose regimen in inverse proportionality. Thus the pre, peri and pan-gestational losses increased with increase in dose and times of exposure in cypermethrin treated groups (Table 1).
Table 1: Data obtained for corpus luteal, implantations and live fetal count recovered and Calculated values of Pre, Peri and pan-gestational Losses in F0 mice following gestational exposure to various doses of cypermethrin

<table>
<thead>
<tr>
<th>Groups</th>
<th>Leutial count (Dams)</th>
<th>Corpus luteia / Female ±MSE</th>
<th>Total No. of Implants (Resorptions)</th>
<th>No. of implants/Female±MSE</th>
<th>Live fetuses/litter</th>
<th>Percent Pre-implantational losses</th>
<th>Pregnancy index</th>
<th>Post implantational losses/litter</th>
<th>Pan-gestational losses/litter</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>180 (20)</td>
<td>9±0.29</td>
<td>174 (3)</td>
<td>8.7±0.22</td>
<td>8.55</td>
<td>3.33</td>
<td>96.67%</td>
<td>1.67</td>
<td>5.0</td>
</tr>
<tr>
<td>SINGLE 3.2mg</td>
<td>188 (20)</td>
<td>9.4±0.50</td>
<td>176 (4)</td>
<td>8.8±0.35</td>
<td>8.6</td>
<td>6.38</td>
<td>93.62%</td>
<td>2.13</td>
<td>8.51</td>
</tr>
<tr>
<td>TRIPLE 3.2mg</td>
<td>172 (20)</td>
<td>8.6±0.23</td>
<td>156 (6)</td>
<td>7.8±0.29</td>
<td>7.55</td>
<td>9.30</td>
<td>90.70%</td>
<td>3.49</td>
<td>12.79</td>
</tr>
<tr>
<td>SINGLE 6.4mg</td>
<td>170 (20)</td>
<td>8.5±0.17</td>
<td>152 (5)</td>
<td>7.6±0.24</td>
<td>7.35</td>
<td>10.59</td>
<td>89.41%</td>
<td>2.94</td>
<td>13.53</td>
</tr>
<tr>
<td>TRIPLE 6.4mg</td>
<td>176 (20)</td>
<td>8.8±0.26</td>
<td>156 (7)</td>
<td>7.8±0.31</td>
<td>7.5</td>
<td>11.36</td>
<td>88.64%</td>
<td>3.98</td>
<td>15.34</td>
</tr>
<tr>
<td>SINGLE 12.8mg</td>
<td>196 (20)</td>
<td>9.8±0.48</td>
<td>172 (6)</td>
<td>8.6±0.26</td>
<td>8.1</td>
<td>12.24</td>
<td>87.75%</td>
<td>3.06</td>
<td>15.31</td>
</tr>
<tr>
<td>TRIPLE 12.8mg</td>
<td>192 (20)</td>
<td>9.6±0.41</td>
<td>164 (12)</td>
<td>8.2±0.28</td>
<td>7.6</td>
<td>14.58</td>
<td>85.42%</td>
<td>6.25</td>
<td>20.83</td>
</tr>
</tbody>
</table>
Table 2: Fetal Density of F0 fetuses following gestational exposure to various doses of cypermethrin

<table>
<thead>
<tr>
<th>Exposure Groups</th>
<th>Fetal Density (mg/ml) ± SD (No. of fetuses)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>1.099±0.192mg/ml (171)</td>
</tr>
<tr>
<td>SINGLE 3.2mg</td>
<td>1.1463±0.077mg/ml (172)</td>
</tr>
<tr>
<td>TRIPLE 3.2mg</td>
<td>1.148±.237mg/ml (150)</td>
</tr>
<tr>
<td>SINGLE 6.4mg</td>
<td>1.138±0.159mg/ml (147)</td>
</tr>
<tr>
<td>TRIPLE 6.4mg</td>
<td>1.084±.202mg/ml (149)</td>
</tr>
<tr>
<td>SINGLE 12.8mg</td>
<td>1.029±0.135mg/ml (166)</td>
</tr>
<tr>
<td>TRIPLE 12.8mg</td>
<td>1.004±0.072mg/ml (152)</td>
</tr>
</tbody>
</table>

Fetal density

Fetal density was taken as an indirect measure of the extent of ossification in the fetal skeleton. The data obtained shows an overall significant (P<0.05) variation. Surprisingly it was noted that the density remained higher than control in both sub-groups in 3.2mg/kg and single exposure sub-group in 6.4mg/kg exposure group. On the other hand triple exposure sub-group of 6.4mg/kg and both single and triple exposure sub-groups of 12.8mg/kg group a decline in fetal density to that of the control was noted. However in all three cypermethrin (i.e. 3.2, 6.4 and 12.8 mg/kg) treated groups a general decline was noted in the triple exposure than that of their respective single exposure sub-groups (Table 2).

Table 3: Actual number of F0 fetuses in each particular abnormality recovered from various experimental groups a: Number of abnormal fetuses (Total number of fetuses recovered)

<table>
<thead>
<tr>
<th>Abnormalities</th>
<th>Groups</th>
<th>3.2mg/kg</th>
<th>6.4 mg/kg</th>
<th>12.8mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Single Treatment</td>
<td>Triple Treatment</td>
<td>Single Treatment</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>0(171)*</td>
<td>1(172)</td>
<td>3(150)</td>
<td>2(147)</td>
</tr>
<tr>
<td>Hydrocephaly</td>
<td>0(171)</td>
<td>2(172)</td>
<td>3(150)</td>
<td>3(147)</td>
</tr>
<tr>
<td>Un-detached pinnae</td>
<td>0(171)</td>
<td>5(172)</td>
<td>7(150)</td>
<td>4(147)</td>
</tr>
<tr>
<td>Epininate ears</td>
<td>0(171)</td>
<td>0(172)</td>
<td>0(150)</td>
<td>0(147)</td>
</tr>
<tr>
<td>Skewed neck</td>
<td>0(171)</td>
<td>0(172)</td>
<td>1(150)</td>
<td>0(147)</td>
</tr>
<tr>
<td>Meromelia</td>
<td>0(171)</td>
<td>0(172)</td>
<td>1(150)</td>
<td>2(147)</td>
</tr>
<tr>
<td>Extra dactly</td>
<td>0(171)</td>
<td>0(172)</td>
<td>0(150)</td>
<td>1(147)</td>
</tr>
<tr>
<td>Drooping wrist</td>
<td>1(171)</td>
<td>2(172)</td>
<td>2(150)</td>
<td>3(147)</td>
</tr>
<tr>
<td>Round back</td>
<td>1(171)</td>
<td>2(172)</td>
<td>3(150)</td>
<td>4(147)</td>
</tr>
<tr>
<td>Hemoregia</td>
<td>1(171)</td>
<td>3(172)</td>
<td>2(150)</td>
<td>5(147)</td>
</tr>
<tr>
<td>Torted hid limb</td>
<td>0(171)</td>
<td>1(172)</td>
<td>2(150)</td>
<td>2(147)</td>
</tr>
<tr>
<td>Forked paws</td>
<td>0(171)</td>
<td>0(172)</td>
<td>0(150)</td>
<td>0(147)</td>
</tr>
<tr>
<td>Flipper limbs</td>
<td>0(171)</td>
<td>0(172)</td>
<td>0(150)</td>
<td>0(147)</td>
</tr>
<tr>
<td>Kinky tail</td>
<td>0(171)</td>
<td>0(172)</td>
<td>0(150)</td>
<td>0(147)</td>
</tr>
</tbody>
</table>
Fetal morphology

Various morphological abnormalities observed in different cypermethrin exposed sub-groups were: 1) microcephaly, 2) hydrocephaly, 3) un-detached pinnae, 4) epinnate ears, 5) skewed cervical region of spine, 6) meromelia, 7) fore limb extra dextly, 8) drooping wrists, 9) round back, 10) abdominal hemoregia, 11) torted hind limbs, 12) forked paws, 13) flipper shaped limbs and 14) kinky tail (Table 3 and Fig. 3.1-3.6).

Fig. 3.1 and 3.2: Fetuses from Control (A); and 3.2 mg/kg triple exposure sub-groups group (B) a: hydrocephaly, b: meromelia c: drooping wrists, d: abdominal hemoregia, e: round back, f: kinky tail, g: torted hind limbs

Fig. 3.3 and 3.4: Fetuses from Control (A); and 12.8 mg/kg triple exposure sub-group (B and D); C: a closer view of selected area from B, E: a closer view of selected area from D a: extra dextly, b: forked paw c: flipper limbs, d: epinnate ear
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Fig., 3.5: Fetuses from Control (A); and 6.4 mg/kg triple exposure sub-group (B and C) and 3.6: closer views of cranial area from A and B of fig. 3.5.  
*a:* microcephaly ,  
*b:* hydrocephaly  
*c:* skewed cervical region of spine ,  
*d:* normal ear pinna,  
*e:* un-detached pinna

**Fetal crown rump length**

Crown rump length is an indication of fetal growth rate. Intra uterine growth retardation is shown as comparison of means of crown rump length of cypermethrin exposure groups to that of the control group (Fig. 4). Analyses of variance indicate that among the three cypermethrin treated groups (i.e., 3.2, 6.4 and 12.8mg/kg); there was no significant variation within the single and/or triple exposure categories. On the other hand the three cypermethrin exposed groups showed significant differences ($P<0.05$) with that of control at both levels of exposures (i.e., single, and triple). Analyses of the means of the respective subgroups of cypermethrin treated groups with that of the control through “Duncan’s Multiple Range Test ” also indicate the significant variation ($p \leq 0.05$), as it has been indicated by means of asterisks in Fig. 4.
Fig. 4: Average crown-rump length of F0 fetuses in various cypermethrin treated sub groups to that of the control
+bars: SEM; *: mean values differing significantly from control; S: Single exposure; T: Triple Exposure; 3.2, 6.4 and 12.8: respective mg/kg dose groups

**Head circumference**

A similar trend, as seen in fetal crown rump length, was observed in fetal head circumference analyses. Analysis of variance of the single and triple exposure subgroups separately; with that of the control, indicate a significant variation in the data (p<0.05). The posthoc analysis of the means have shown significant variations between control and 12.8mg/kg single exposure sub-group; while 6.4 and 12.8mg/kg sub-groups differed significantly with that of the control in triple exposure category (Fig., 5).
Fig5: Average head circumference in F0 fetuses from various cypermethrin treated sub-groups and the control group
+bars: SEM; *: mean values differing significantly from control; S: Single exposure; T: Triple Exposure; **3.2, 6.4 and 12.8: respective mg/kg dose groups

DISCUSSION

There are only a few studies pertaining embryo toxicology dealing directly with the embryonic exposure of cypermethrin (Gupta, 1990; Farag et al., 2007; Uggini et al., 2010). Thus the results obtained in this investigation are hardly comparable to the previously available literature. The present study was aimed to discover the toxicological effects of cypermethrin in relation with pregnancy, pre, peri and pan-gestational losses and to report all sorts of developmental abnormalities at morphological and morphometric levels.

The estimation of LD$_{50}$ was a laborious job. But it was done due to the following reason.
1. Although the values of LD$_{50}$ for mice are available in literature. However there exist a wide disparity in these reported values of LD$_{50}$ for mice for example; 50mg/kg body weight (Luty et al., 2000) and 25mg/kg body weight (Varshneya et al., 1992).
2. All such available values were confined to the males or non-pregnant females. In many cases the available literature does not specify the value of LD$_{50}$ in relation to sex. Moreover there exist no such values in literature dealing especially with the pregnant mice.

Keeping in view of these constrains it was decided that a careful estimation of LD$_{50}$ in pregnant mice should be conducted for the selection of motherly safe dose profile. Results indicate that the value of LD$_{50}$ for
Cypermethrin in pregnant mice remained 64mg/kg body weight. This is considerably higher than the already reported value that is 50mg/kg for non-pregnant mice (Luty et al., 2000). The natural phenomenon of suppression of immunological and humoral responses must have enhanced toleration limits towards this toxic environmental chemical during pregnancy that seems to be responsible for this increase in LD50 value (Fessler, 2001; Calder et al., 2006). An embryo is kept as an allo-graft in mammals and natural defense mechanisms have to be suppressed to avoid any damage to them by maternal immunological responses. Due to this partial suppression of the immune system pregnant female mice may have tolerated higher toxic doses of cypermethrin than the males and non-pregnant females.

Dose and extent of exposure (single or triple) related increased pre-implantation losses that have led to a decline in pregnancy index in cypermethrin treated mothers to that of the control group was considered as a direct embroycidal impact of cypermethrin. As the results show (Table 1) that this increase in pre-implantational losses was clearly dose dependent, it seems partly because of an increased embryonic death (before or just after implantation - leading to complete resorptions in such cases) in a dose dependant manner and partly because of an interference in the process of implantation as cypermethrin has already been accepted as a strong endocrine disruptor of the sex steroids (Waters et al., 2001; Chen et al., 2002; McCarthy et al., 2006) and hormones of gestation particularly LH (Elbetieha et al., 2001).

Fetal resorptions were considerably increased with the increase in exposure dose strength (3.2, 6.4 and 12.8mg/kg). Slight increase was noted in triple exposure sub-groups in 3.2 and 6.4 mg/kg dose levels then there respective single exposure sub-groups, while in 12.8mg/kg group the percent fetal resorption was more than double in the triple exposure sub-group than that of the single exposure sub-group. This finding clearly suggest that higher dose (i.e. 12.8mg/kg (20% of the LD50)) with multiple exposures are far more embryo lethal than the low doses (3.2 and 6.4mg/kg (10 and 5% of LD50)) in the similar experimental conditions (Table 1). On the whole the pregnancy index and fetal resorption data clearly indicating that cypermethrin is highly embryo toxic that interferes with the process of embryonic implantation and intrauterine fetal development. In this context Rutledge, (1997) had argued that chemicals adversely affect the early conceptus causing in-utero mortality and developmental abnormalities. Further more susceptibility of the post-fertilization period differs from exposures of sperms (Elbetieha et al., 2001) or eggs as the later produces massive pan-gestational losses with lesser number of fetal anomalies. In contrast, similar exposure at pre-gastrulational stage induces peri-implantational and pan-gestational death along with various fetal anomalies (Rutledge, 1997). The period of exposure (GD6-12) of this study begins at the end of the critical period of implantation (i.e. GD4.5-6) (Kaufman & Bard, 1999) that has logically to be followed by gastrulation thus the results obtained in terms of co-gestational losses and developmental abnormalities seems justified.

Fetal density was regarded an indirect measure of the extent of ossification. Surprisingly highest fetal density (1.148mg/kg) was noted in 3.2mg/kg triple exposure sub-group while lowest (1.004mg/kg) in 12.8mg/kg triple exposure subgroup. Results indicate that multiple low dose (3.2mg/kg = 5%...
of the LD$_{50}$) gestational exposures might have led to an enhanced Ca$^{++}$ deposition whereas the multiple high dose (12.8mg/kg = 20 % of the LD$_{50}$) have in inverse impact upon Ca$^{++}$ deposition. As cypermethrin is a proven estrogenic agonist (Chen et al., 2002; McCarthy et al., 2006) at low dose multiple exposures it might have led to an enhanced osteocyte differentiation in fetuses leading to an above normal Ca$^{++}$ deposition. The inverse trend in high dose group is clearly due to ostioblastic necrosis and lesser retention of Ca$^{++}$ in the fetal bones (Table 2).

Crown Rump (CR) Length comparison is an important parameter that indicate intrauterine growth retardation in response to the fetal exposure to a noxious environmental chemical substance (like insecticides). Results obtained in present study indicate a dose dependent decline in fetal growth rate as compared with the control group. Multiple exposures even at 3.2mg/kg (i.e. 5% of LD$_{50}$) produced big decline in CR length indicating that the repeat exposures are much more dangerous for in utero fetal growth (Fig4). The results are comparable with similar previous studies (Ahmad & Asmatullah, 2007). Measurement of fetal head circumference is another parameter that along with the general retardation in fetal growth does also indicate the extant of fetal brain growth retardation (Ahmad & Asmatullah, 2007). The results obtained in this regard have also shown the comparable trend as obtained for CR length (Fig5).

The available literature on the fetomorphic derangements attributable to cypermethrin exposure is relatively skimpy (Gupta, 1990; Farag, et al. 2007; Uggini, et al. 2010). However various feto-morphological abnormalities were observed in this study that include microcephaly, hydrocephaly, un-detached pinnae, epinate ears, skewed neck, meromelia, extra dextly, drooping wrist, round back, hemoregic spots, tortured hid limb, forked paws, flipper limbs and kinky tail (Fig 3 and Table 3).

Conclusively cypermethrin was found to the highly toxic to the developing fetuses on dose levels as low as 5% of the LD$_{50}$ (3.2mg/kg) for the mothers. Along with general in utero growth retardation, it has also led to a significant increase in pan-gestational losses along with various feto-morphological abnormalities.

REFERENCES


