Prenatal zestoretic exposure ameliorates developmental landmarks in rat offspring

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Abstract

Zestoretic, a combination of lisinopril and hydrochlorothiozide used as anti-hypertension drug prescribed even during pregnancy. Role of zestoretic on the developmental landmarks of young ones exposed prenatally are yet to be established. Hence, the present investigation initiated to test in Wistar rats. Three groups of inseminated female rats were oral gavaged with zestoretic (lisinopril varied concentrations + hydrochlorothiozide 12.5mg) 25, 50 and 100 mg/Kg body weight (BW) on gestation days 7, 9, 11 and 13, whereas control group with double distilled water. Clinical toxicity found in few pups from 50 and 100 mg/Kg BW received rat shown aggressive behavior during experimentation. The developmental landmarks, such as pinna attachment, ear opening, fur development, eye opening, upper and lower incisor eruption, crown rump length, vaginal opening and testes descend were measured periodically on their postnatal days in all the young ones. The number of live pups in zestoretic 50, 100 mg/Kg BW administered females are significantly less (P<0.001) from control. Comparatively among the groups, sex ratio of male and female offspring is not changed. The BW of young ones on postnatal day (PND) 1, 7, 14 and 21 are significantly (P<0.001) decreased in experimental groups. Decreased survival index of pups exposed to zestoretic 50; 100 mg/Kg BW was reported. Recorded, delay in pubertal onset in females (vaginal opening) and testicular descent in males of experimental groups. Crown rump length in experimental offspring is significantly (P<0.05) decreased from controls in a dose dependent manner on PND 1, 7, 14 and 21. No change has been recorded with anogenital distance, pinna detachment, eye and ear opening, fur development, lower and upper incisor eruption, experimental offspring when compared to controls. The present study establishing the interference of prenatal zestoretic exposure on some of the developmental landmarks in the offspring of rats.

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1. INTRODUCTION

Conventional procedures including usage of common medicine without prescription at critical stages including during pregnancy may damage the health and sometimes even for life. In developing and third world countries it is very common that medicine has been used by innocent women during pregnancy without consultation with medical practitioner which may leads to congenital defective babies (Banhidy et al., 2005; Punam et al., 2009). Drugs to reign blood pressure are used by men and women, even during pregnancy either by intentionally or unintentionally (Lindheimer et al., 2010; Scantlebury et al., 2013). In men the common medicine includes that govern blood pressure may show many side effects like diminish in spermatogenesis and debacle copulation (Eisenberg et al., 2015; David et al., 2017). The embryonic exposure of these chemicals by foetus/new-borns are somehow divergent, may cause malformation in the developing embryo while gestation or after accouchement and sometimes at adult stage. Brezina et al (2012) explained the effects caused by pharmaceutical medications on male fertility. Besides these, recreational drugs on male fertility were also well documented (Kulkarni et al., 2014). Similarly, the reason for deteriorated male reproductive health is well presented by Sunil kumar et al (2019).

In many cases, malformation may not be identified, baby look like normal and grown up. But at the adult stage malformations including infertility can be identified. However, the reason for increased impotency of males and significant fall in reproduction abilities of women is notable today (Grimm et al., 1997; Peter et al., 2020). This kind of aberrations must be addressed with proper evidence. It is needed especially for woman to prevent the usage of drugs that affect the growth of foetus and young ones during gestation and lactation (Deborah et al., 2005; Sachdeva et al., 2009).

Zestoretic, a drug commonly used to treat high blood pressure (hypertension), and it is amalgamation of
lisinopril and hydrochlorothiazide (HCTZ). No scientific reports are available on zestoretic induced reproductive aberrations in males and females. Moreover, no reports found for its toxicity, malformations, alteration in the developmental landmarks of infants exposed administered to zestoretic prenatally. The present investigation hypothesized that zestoretic alters the developmental landmarks in offspring of Wistar rats upon exposure during pregnancy along with behavioural aspects of dams.

2. MATERIALS AND METHODS

2.1. Test animals and their maintenance

The fact-finding animal model i.e., Wistar albino rats (30 rats; 60 days old; 195 ± 5 g body weight) were selected as test animal in this study and procured from at Sri Venkateswara Traders, Bengaluru, India. Test rats domiciled in well ventilated (12 h:12 h light: dark cycle), clean and controlled room temperature (25 ± 2°C; relative humidity of 50 ± 5%). All experimental rats feed ad libitum with certified rodent feed (Sri Venkateswara Traders, Bengaluru, India) and water. The trails executed in accordance with the guidelines of the Indian statutory body (CPCSEA: Committee for the Purpose of Control and Supervision on Experiments on Animals) (CPCSEA, 2003). Further, fulfilled the guidelines of the safe keeping and use of laboratory animals (NRC, 1996) and endorsed by the Institutional Animal Ethical Committee, Vemana University, Kadapa, A.P., India (No.YVU/IEAC/PRR/13/2017).

2.2. Chemicals and Equipment

The tryout chemical used in the current investigation is zestoretic, it is a combination of both diuretic HCTZ and angiotensin converting enzyme (ACE) inhibitor lisinopril. Lisinopril (zestril) is regular medicine available in pahrama located in, California, USA, HCTZ from life pharma, Kadapa, A.P., India. Lisinopril, a synthetic peptide derivative, chemically it is (S)-1-[N2-(1-carboxy-3-phenylpropyl)-L-lysyl]-L-proline dihydrate (Figure 1A). Lisinopril physically a white to off-white crystalline fine powder with a molecular mass (MW) of 441.53 g/mol and is soluble in water, methanol (moderate) and ethanol (partial). HCTZ is 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1dioxide (Figure 1B). HCTZ a crystalline white or practically white powder with MW of 297.72g/mol and its solubility is partial in water and high in NaOH (sodium hydroxide) solution. Sodium chloride and other chemicals used in this study were purchased from Hi media Pvt. Ltd., India. Oral administration of zestoretic by using oral gavage, type AQRA, HARVARD apparatus, Maharashtra. Measurement of crown rump length by using SSEA stainless iron nickel vernier caliper and body mass of rat is weighed by electronic balance, Type AY220, SHIMADZU Corporation, Japan.

![Figure 1. Structure of Lisinopril (A) and Hydrochlorothiazide (B).](image-url)
Zestoretic exposure has shown no clinical signs in the dams or F1 progeny. Only observed signs that are different from normal are aggressive behavior in few dams which are exposed to 50 and 100 mg zestoretic/Kg BW. The number of live pups per rat significantly (p<0.001) decreased in groups exposed prenatally to zestoretic when compared to controls (Table 1). The litter size is reduced in the 50 and 100 mg zestoretic exposed groups, but the male and female sex ratio is comparable with control group. On PND 1, 7, 14 and 21 the body mass of offspring exposed to zestoretic 100 mg/Kg BW was significantly (p<0.0001) decreased when compared to control group (Table 2). The pups of other two experimental groups (zestoretic 25 and 50 mg/Kg BW exposed) were shown slight decrease in the body mass/weight on PND 1 and 7, but are in line with controls on PND 14 and 21. However, the weight loss in offspring is dose dependent in the experimental groups over the controls. No significant change has been recorded in anogenital distance, whereas survival index is reduced on PND 4 and 21 in zestoretic 50 and 100 mg/Kg BW exposed pups compared to controls (Table 3).

Figure 3. Testis descent of the pups exposed to zestoretic prenatally on postnatal day 1, 7, 14 and 21. Z= zestoretic; BW=body weight.

Table 2. Body weight (gm) of pups exposed to zestoretic during transplacental and perinatal development on PND 1, 7, 14, and 21.

<table>
<thead>
<tr>
<th>PND</th>
<th>Control</th>
<th>25mg/Kg BW</th>
<th>50mg/Kg BW</th>
<th>100mg/Kg BW</th>
<th>F-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.17±0.097</td>
<td>6.16±0.062</td>
<td>5.79±0.69a</td>
<td>5.69a</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>7</td>
<td>11.6±(1.13)</td>
<td>11.45±0.07</td>
<td>11.13±0.14a</td>
<td>10.41±0.04</td>
<td>P&gt;0.001</td>
</tr>
<tr>
<td>14</td>
<td>18.63±(1.63)</td>
<td>18.57±0.047</td>
<td>18.47±0.048</td>
<td>17.56±0.04</td>
<td>P&gt;0.001</td>
</tr>
<tr>
<td>21</td>
<td>23.67±(2.03)</td>
<td>23.57±0.054</td>
<td>23.52±0.05</td>
<td>22.68±0.04</td>
<td>P&gt;0.001</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 8 individuals. BW=body weight.

3. RESULT AND DISCUSSION

Table 1. Number of live pups/rat and sex ratio of offspring of rats exposed to zestoretic during embryonic development.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>25mg/Kg BW</th>
<th>50mg/Kg BW</th>
<th>100mg/Kg BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of pups/rat</td>
<td>11.75±0.25</td>
<td>11.75±0.25</td>
<td>10.9±0.40</td>
<td>8±0.409</td>
</tr>
<tr>
<td>Male: Female pups</td>
<td>26:21</td>
<td>23:24</td>
<td>24:18</td>
<td>17:15</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 8 individuals. BW=body weight.

Values in parentheses are percent change from control. P<0.05 was considered as significant.

The low survival index was recorded in 100 mg zestoretic/Kg BW exposed pups on PND 4 (75%) and 21
(62.5%). Crown rump length is significantly decreased (P=0.0001) in zestoretic exposed pups when compared to control group on PND 1, 7, 14 and 21 (Table 4).

### Table 3. Trans-placental exposure of zestoretic on anogenital distance and survival index of rat pups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>25mg/Kg BW</th>
<th>50 mg/Kg BW</th>
<th>100mg/Kg BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anogenital distance (cm)</td>
<td>0.41±0.02</td>
<td>0.41±0.02</td>
<td>0.41±0.02</td>
<td>0.4±0.01</td>
</tr>
<tr>
<td>(male pups on PND 1)</td>
<td>P=0.5983</td>
<td>P=0.5983</td>
<td>P=0.5983</td>
<td>P=0.5983</td>
</tr>
<tr>
<td>Anogenital distance (cm)</td>
<td>0.35±0.018</td>
<td>0.32±0.018</td>
<td>0.32±0.016</td>
<td>0.32±0.016</td>
</tr>
<tr>
<td>(female pups on PND 1)</td>
<td>P=0.6845</td>
<td>P=0.857</td>
<td>P=0.3506</td>
<td>P=0.3506</td>
</tr>
<tr>
<td>Survival index of pups (%)</td>
<td>47/47</td>
<td>47/47</td>
<td>35/42</td>
<td>24/32</td>
</tr>
<tr>
<td>(PND 1)</td>
<td>(100)</td>
<td>(82.50)</td>
<td>(75.0)</td>
<td></td>
</tr>
<tr>
<td>Survival index of pups (%)</td>
<td>45/47</td>
<td>42/47</td>
<td>32/42</td>
<td>20/32</td>
</tr>
<tr>
<td>(PND 21)</td>
<td>(95.74)</td>
<td>(89.36)</td>
<td>(71.42)</td>
<td>(62.50)</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 8 young rats. BW: Body Weight. Values in parenthesis are percent change from controls. P<0.05 was considered as significant.

No considerable change has been reported in developmental landmarks like pinna detachment, fur development, lower and upper incisor eruption, eye and ear opening in the young ones exposed prenatally to zestoretic when compared to control (Table 5). Significant increase (P<0.0001) in the mean numbers of preputial separation was observed in 50 (43.5±0.534) and 100 (45±0.756) mg zestoretic/Kg BW exposed pups when compared to control and 25 mg zestoretic/Kg BW exposed groups (Table 5). The delay in mean days for testes descent of experimental male pups (25, 50 and 100 mg/Kg BW exposed; 27.25±0.463, 28±0.756 and 29.12±0.641 respectively) is significant (P<0.001) when compared to control rats (26.25±0.463) (Table 5, Figure 4). Compared to controls (34.37±0.517) female pups exposed prenatally to zestoretic (25, 50 and 100 mg/Kg BW; 35.75±0.463, 36.62±0.517 and 37.87±0.641 respectively) showed significant (p<0.001) delay in mean days of vaginal opening (Table 5, Figure 4).

### Figure 4: Vaginal opening of the pups exposed to zestoretic prenatally on postnatal day 1, 7, 14 and 21. Z= zestoretic; BW=body weight.

Zestoretic is an antihypertensive drug used to treat blood pressure. In the current study we experimented the developmental abnormalities and toxicity of zestoretic at viability of pups, male and female ratio in transplacental ACE inhibitors orally administered groups compared to the control (Shrimet al., 2005; Bhatia et al., 2013; Hetterich et al., 2014). Annette et al (2009) explained about lisinopril (ACE inhibitor) nature and Olesen et al (2001) described diuretics in decreasing the body weight of new borns. In current study, prenatal exposure of zestoretic showed significant (p<0.0001) decrease in the mean body mass/weight of zestoretic 100 mg/Kg BW exposed pups on 1st, 7th, 14th and 21st PND. No modification found in the body mass of zestoretic 25 and 50 mg/Kg BW exposed offspring on PND, 1, 7, 14 and 21. Survival index of pups on 4th and 21st PND, and in the survival rate of young ones exposed to zestoretic prenatally found lower.

No change has been recored in anogenital distance by prenatal zosteretic exposure and are similar to the controls in both genders of offspring. Compared to control offspring, significant decline (p<0.0001) in crown lump length and survival index on 1st, 7th and 21st PND is reported in this study. Aslo found significant decrease (p<0.0001) in the survival rate of offspring exposed prenatally to zestoretic. Change is not significant in pinna detachment, ear opening, fur development, upper and lower incisor eruption, eye and ear opening in the young ones exposed prenatally to zestoretic. Reduced litter size and decline in number of male and female sex ratio was reported in the present study. Reduced male and female ratio from control compared to increase the dose concentration, correlating with decrease live pups number/chance, increased trans-placental fetal toxicity of zosteretic towards rise of resorptions and less number of implantations in a dose dependent manner and which is in concurrence with other studies (Rosenthal and Oparil, 2002). There is a decline in
lower incisor eruption of experimental groups from the control young ones. Kwak et al (2012) reported ACE inhibitors had significantly decrease serum dehydroepiandrosterone (DHEA) level and lower level of serum androstenedione (Westwood, 2008). Transplacental exposure of zestoretic 25, 50 and 100 mg/Kg BW caused significant decrease (p<0.0001) in delaying the testicular descent of male offspring when compared to the control. Similar results are reported in rat offspring exposed prenatally to biochanin-A and baicalein (Sridevi et al., 2016).

**Table 5.** Effect of trans-placental and weaning exposure of zestoretic on developmental landmarks of rat pups,

Values are mean ± SEM of 8 young rats. BW: body weight. Values in parenthesis are percent change from controls. P<0.05 was considered as significant

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control 25 mg/Kg BW</th>
<th>50 mg/Kg BW</th>
<th>100 mg/Kg BW</th>
<th>F-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinna detachment</td>
<td>5.25±0.13</td>
<td>3.25±0.13</td>
<td>3.37±0.13</td>
<td>p=0.9497</td>
</tr>
<tr>
<td>Lower incisor eruption</td>
<td>3.37±0.18</td>
<td>3.37±0.18</td>
<td>3.37±0.18</td>
<td>p=0.9234</td>
</tr>
<tr>
<td>Fur development</td>
<td>7.54±0.18</td>
<td>7.62±0.18</td>
<td>7.54±0.1</td>
<td>p=0.9599</td>
</tr>
<tr>
<td>Testes descent</td>
<td>26.25±0.14</td>
<td>28±0.75</td>
<td>29.12±0.14</td>
<td>p=0.0001</td>
</tr>
<tr>
<td>Vaginal opening</td>
<td>35.75±0.33</td>
<td>36.62±0.12</td>
<td>37.87±0.12</td>
<td>p=0.0001</td>
</tr>
<tr>
<td>Preputial separation</td>
<td>42.38±0.67</td>
<td>43.56±0.53</td>
<td>45±0.75</td>
<td>P=0.0001</td>
</tr>
</tbody>
</table>

50, 100 mg zestoretic/Kg BW displays significant rise (p<0.0001) in the number of days for vaginal opening in female rat pups. In process of factfinding results supports that the increased days for vagina opening in zestoretic exposed female young rats may be due to early release of pubertal hormones promoted by zestoretic. Moreover, there are no major change observed in the body mass/weight of offspring exposed to 25 mg zesteric prenatally. But, zestoretic 50 and 100 mg/kg BW prenatal exposure resulted to significant change (p<0.0001) in preputial separation in young ones. Joel et al (2007) explained additional risk of ACE inhibitor cause malformation of cardiovascular system and central nervus system leads to foetal toxicity. Using of diuretics during gestation period were at increased risk of premature delivery and rise risk of malformations in the progeny (Olesen et al., 2001).

4. CONCLUSION

The effect of zestoretic on growth and development of the gonadal tissue in both sex offspring of rats which may alters the levels of gonadal hormones. For all the observed abnormalities, though there is no direct evidence of zestoretic induced abnormalities in humans at the recommended dosage, developmental abnormalities are reported in rats in the present study. So, the current data may not be extrapolated to humans. However, the pregnant mothers should be aware of these orally administration of zestoretic in order to overcome the infertility and impotence complication in offspring. Future studies with longer duration of zestoretic exposure is necessary to better account for its effects and possible mechanisms as an endocrine disruptor to the reproductive system in both male and female. Thus, in spite the uncritical recommendation on the use of zestoretic and it is advised to avoid the usage of complicated medicine during pregnancy.

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REFERENCES


