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Possible Mechanism Involved in Causing Oligohydramnios Following ACEI & Guldaudi Extract Administration in Mice.

KEYWORDS: Fetus, growth retardation, congenital malformation, oligohydramnios.

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ABSTRACT *ACE Inhibitors have been found to be teratogenic in man (Moore et al.,2008)(1). In the present study ACE Inhibitor (Lisinopril) and extract of Guldaudi which is supposed to have ACE Inhibitor like activity, were administered to mice throughout gestation period to understand the mechanism of teratogenicity, in the dose of 2mg/100g of Lisinopril and 50mg/100g of Guldaudi extract. Various malformations were found i.e. (a) stunting in size b) malformations of limbs and toes (c) and haemorrhages on general body surface. Quantity of amniotic fluid in the treated group of lisinopril and Guldaudi was found less than the control and on 18th days of intrauterine age the average quantity in the control and treated sacs was 233.75 µl, 148.15 µl and 154.84 µl respectively.The significant change in the amniotic fluid quantity suggest oligohydramnios causing various malformations.*

Introduction:
The first ACE inhibitor when discovered it was reported as a natural peptide isolated from the venom of Brazilian snake's (Ferreira et al., 1970; Ondetti et al., 1971) (2,3). After that this peptide used as a model system and specific ACE inhibitors such as captopril and enalapril have been synthesized and used in the treatment of hypertension. But it was also reported that, when ACE inhibitors are used during the pregnancy they cause the congenital malformations.

Various plant products (Ariyoshi 1993; Park et al., 1996; 1998; Matusi et al., 1993; Yamamoto 1997; Fujita et al., 2000) (4,5,6,7,8) which are known to contain ACEI like activity. The Chrysanthemum indicum species, a Compositae family plant, is also known to contain ace inhibitor like activity in their flowers and used to treat the hypertension; (J. Kim et al., 2003) (9) therefore it was conceived to work on this topic to find out teratogenic effect of lisinopril as ACE inhibitor and Chrysanthemum Indicum during the pregnancy.

Material and Methods:
A total of 18 Swiss Albino female mice were used for this study. Mice were obtained from the central animal house of Institute of Medical Sciences, Banaras Hindu University. These were primygravida having an average age of 50 days and body weight ranging between 25- 45 grams. These mice were kept in separate individual cages in room with temperature maintained at 75°F & 50% humidity and a light & dark cycle of 12:12 hours.

Female mice during proestrus were kept with males of the same stock in cages overnight. In the following morning the vaginal smear was checked for the presence of sperms. Animal with sperm positive smear was labeled as at day Zero of pregnancy. Weight of the pregnant mice was recorded daily and the increase in weight was studied. The pregnant mice were divided into three groups.

The first group was control and other two groups were exposed continuously, from day zero of pregnancy till parturition, to 2 mg/100 g dose of ACE inhibitor (lisinopril) and 50mg/l00g body weight Guldaudi extract. There were 5 female mice in the control group, and 6 female mice in 2 mg dose group of lisinopril or 7 female mice in Guldaudi extract dose group of 50mg/100g. Throughout the experiment, guidelines of laboratory animal care were strictly followed. The lisinopril and Guldaudi extract were administered in the pregnant mice by oral route using an improvised no-18G needle (blunt tip) fitted to a tuberculin syringe. Normal saline was used in the control group.

Collection of fetus:
On day 18 (at 8 a.m.) of gestation, in order to avoid natural birth, each pregnant mice from all the experimental groups was sacrificed with overdose of ether anaesthesia. Uterine horns were exteriorized after opening the abdomen and pups were examined in situ in the uterine horns for sites of resorptions and viable fetuses. The fetuses with placenta after removal from the horns, together with intact amniotic sac were weighed and blotted dry and weighed again individually without amniotic fluid. The difference in weights was considered the quantity of amniotic fluid in the sac. The fetuses were examined under the dissecting microscope for external malformations. The fetal growth parameters recorded, CRL in cm, fetal weight in gm, and gross malformations parameters including stunting in size, kinking of tail, exencephaly, absence of calvaria with or without exencephaly etc. Detail histological study of the kidney of the pups was also carried out for recording malformations.

Observations:
Total number of fetuses available for the study was 109 from the three groups of mice. Table- 1 shows the number of pups in each group.

In the present study, when fetal weight was recorded after collection, on day 18 of gestation, showed stunting in the size of pups. As evident from the table -1, the control group fetuses had a mean weight of 1.36 gm, and the mean weight of the fetuses was recorded less than the control, in the treated groups i.e. 1.05 gm in (2 mg /100 gm) of lisinopril group and 1.12 gm in 50 mg /100 gm Guldaudi extract group respectively.

The weight of the pups of all treated groups was less than the control group and the difference was statistically significant, $p<0.001$.

Mean Crown-Rump length on day 18 of gestation, as seen in Table -2, was 2.40 cm in the control and in the treated groups i.e. in (2mg/100g) of lisinopril and 2.19 cm and in 50 mg /100 g Guldaudi extract group was 2.11 cm respectively.

This confirms that the ACE inhibitors (lisinopril and Guldaudi extract) are teratogenic in terms of reducing body weight and size. However, these doses are not lethal.

Observations other than the above were micrognathia, deformities of the limbs like malrotation, amputation at different levels of limbs (Fig-4 & 5).

Volume : 3 | Issue : 4 | April 2013 | ISSN - 2249-555X

Observation by sectioning the kidney of treated pups with lisinopril (2mg /100 gm dose) and guldaudi extract (50mg/100gm dose) at higher magnification.

It was observed that ACEI (2mg lisinopril) treated kidney glomerulus shows afferent and efferent vessels comparatively well demarcated but afferent arteriole is dilated as compare to control (Fig-1). The urinary space is much enlarged in width. The parietal layer of the Bowman's capsule shows the discontinuity at places as if the accumulation of urine in the urinary space (star) was in large quantity and exerting pressure making it rupture (Fig-2). The sections of the proximal and distal convoluted tubules and loop of Henley also appear to be dilated (Fig-2) x 100 and Guldaudi extract treated kidney nephron shows disfiguration (Fig-3). The urinary space (star) is enlarged and parts of the proximal distal convoluted tubules show comparatively dilated (Fig-3). (x 100)

Discussion:
The action of ACEI (Lisinopril) in 2 mg/100 gm dose and Guldaudi flower extract in the 50 mg/100gm dose (which is supposed ACEI like activity) was studied in pregnant mice it was found teratogenic.

In the present study both lisinopril and extract of Guldaudi flower were administered to pregnant mice throughout pregnancy to study their effect on the development of kidney. It was observed that when compared with control, the administration of the two preparations causes renal changes both structurally and functionally. It was anticipated that these two preparations will reach the fetus crossing the placental barrier and may interfere in the development of kidneys of the mice pups causing structural and functional changes.

When these changes of the kidney in lisinopril treated pup were compare with the Photomicrograph of section of a control mice pup kidney at high magnification the glomerulus shows dilated afferent arteriole than efferent arteriole. The urinary space is much enlarged in width. The parietal layer of the bowman's capsule shows the discontinuity at places. The sections of the proximal and distal convoluted tubules and loop of Henley also appear to be dilated. (x 100) while the photomicrograph of section following Guldaudi administration during pregnancy the nephron shows disfiguration. The urinary space is enlarged and parts of the proximal distal convoluted tubules show comparative dilated. (x 100)

Our finding are confirmed by another study was carried out by Mitsuru et al., (1995)(10) i.e. the long term administration of the angiotensin-converting enzyme inhibitor lisinopril on renal arteriols in spontaneous hypertensive rats(SHR) and Wistar-Kyoto rats (WKY) using a morphometric method and vascular cast technique. Rats were treated with lisinopril beginning at 4 weeks of age. At 15 weeks of age, the kidney vessels were fixed when maximally relaxed. Resin was perfused into the right kidney to make a cast of a renal vasculature. The opposite kidney was used for the morphometric study to evaluate structural changes of the vascular wall. The vascular cast study demonstrate a significant reduction in the lumen diameter of the afferent but not the efferent arterioles in SHR compared with those in WKY. In lisinopril -treated rats, the afferent arteriolar lumen diameters significantly smaller in SHR than WKY, suggesting that the the impaired growth of the afferent arteriolar media was involved in narrowed afferent arteriolar in SHR. The presence of significantly smaller media-lumen ratio, greater media cross-sectional area, and larger internal as well as external diameters of the afferent arterioles in treated SHR than in untreated rats suggested that lisinopril treatment normalizes the structure of the afferent arterioles in SHR by vascular reverse remodeling and by inducing media growth.

The effect of lisinopril, an ACE inhibitor, on the structure of afferent and efferent arterioles showed that (1) lisinopril treatment results in a larger lumen diameter in afferent arterioles, without a change in efferent arteriolar diameter in either SHR or WKY, and (2) larger afferent arteriolar diameter with lisinopril treatment is accounted for by a process of reverse remodeling plus an increased amount of vascular media rather than by a preventive action on cellular growth.

In present study larger afferent arteriole diameter indicate the effect of ACE inhibitor in both lisinopril and Guldaudi groups as well as the more structural deformity in guldaudi group.

Exposure of the fetus to ACE inhibitors as antihypertensive agents causes oligohydramnios, fetal death, hypoplasia of the bone of the calvaria (cranial vault)(Fig-5), IUGR and renal dysfunction. During early pregnancy the risk to the embryo is apparently less and there is no indication to terminate a pregnancy. However, because of the high incidence of serious perinatal complications, it is recommended that ACE inhibitors should not be prescribed during pregnancy (Moore and Persaud, 2008)(1). Angiotensin-converting enzyme inhibitor exposure produce reversible oligohydramnios in a pregnancy (Chisholm et al., 1997)(11).

Due to renal dysfunction of fetus, the amount of the urine excreted by fetus into the amniotic fluid /sac would be less in quantity causing reversible oligohydramnios (Chisholm et al., 1997)(11). Severity of oligohydramnios would also be increased due to failure of addition of secretion from the lungs of the pups under going hypoplasia (Crane et al., 1968)(12). The oligohydramnios secondary to renal and lung damage itself is known to be highly teratogenic.

It can be concluded that both lisinopril and Guldaudi due to ACEI effect causes renal structural changes and dysfunction. The kidneys fail to develop to normal size and amount of urine exerted becomes less. The resultant oligohydramnios causes various teratogenicities (moore et al.,2008)(1).

Table- 1: Showing weight of pups of mice after exposure to lisinopril & Guldaudi extract.

Groups	N (109)	Range in g	Mean in g	SD in g	SE in g	Growth Retardation Compare to control
Control	32	1.25-1.55	1.36	0.083	0.014	% less than average
Lisinopril 2mg/100gm	46	.60-1.68	1.05	0.250	0.036	22.90%
Guldaudi Extract 50mg /100g	31	.90-1.30	1.12	0.112	0.033	17.39%

As depicted in Table-1 the control group fetuses had an average weight of 1.36 gm. In treated groups the average weight of fetuses of 2mg lisinopril group, was remarkably less i.e. 1.05 gm. The average weight of Guldaudi group fetuses was also less as compared to control group but more than lisinopril group.

Table- 2: Showing CR length of pups of mice after exposure to different doses of Lisinopril during intrauterine life.

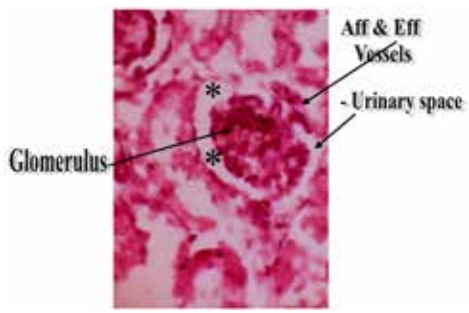
Groups	N (216)	Range in cm	Mean in cm	SD in cm	SE in cm	Growth Retardation Compare to control
Control	32	2.34-2.48	2.40	0.043	.007	% less than average
Lisinopril 2mg/100g	46	1.95-2.05	1.99	0.029	0.004	16.72%

Volume : 3 | Issue : 4 | April 2013 | ISSN - 2249-555X

Guldaudi Extract 50 mg /100g	31	1.90-2.30	2.11	0.116	0.116	11.79%

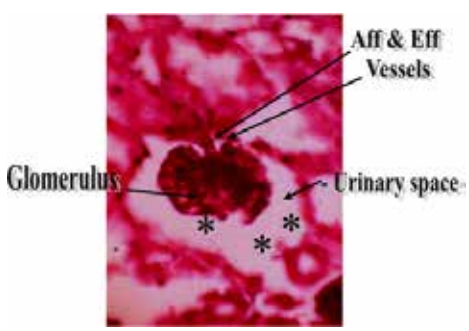
As depicted in Table-2, average Crown Rump (CR) length on day 18 of gestation, as seen in table-2, was 2.40 cm in the control. The average crown rump length of the 2mg ACEI treated groups was less 1.99 cm than the control group P<0.001. In Guldaudi treated group the average CR Length was also less than the control group 2.11 cm.

Fig-1



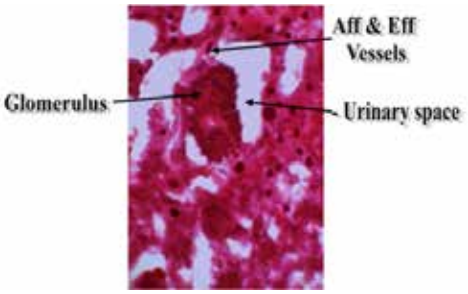
CONTROL KIDNEY
Photomicrograph of section of a control mice pup kidney at high magnification showing the well formed glomerulus surrounded by uniformed urinary space(*). The afferent & efferent vessels going and leaving to the glomerulus are well differentiated. The rest of the field shows proximal & distal convoluted tubules and loop of Henley in different planes. (x 100)

Fig-2



ACEI TREATED KIDNEY
Section through the kidney of a treated pup with lisinopril at higher magnification. The glomerulus shows afferent and efferent vessels but dilated afferent arteriole as compare to control. The urinary space is much enlarged in width. The parietal layer of the bowman's capsule shows the discontinuity at places as if the accumulation of urine in the urinary space was in large quantity and exerting pressure and not getting drained at the required rate therefore urine develop pressure on the visceral and parietal layer of Bowman's capsule. The sections of the proximal and distal convoluted tubules and loop of Henley also appear to be dilated. (100 x)

Fig-3



GULDAUDI TREATED KIDNEY
Photomicrograph of section following Guldaudi administration during pregnancy the nephron shows disfiguration. The urinary space is enlarged and parts of the proximal distal convoluted tubules show comparatively dilated. (x 100)

Fig-4

Gross photograph shows a control mice(C) compared with a ACEI treated pup (T) exposed to 2 mg during full period of gestation intra uterine life. The treated pup shows stunting in size and has conspicuous shape of the snout and head (flat anteroposteriorly) pointed by red arrows & the limbs show malrotation and various anomalies of toes also pointed by red arrow.

Fig-5

Gross photograph shows guldaudi extract treated pup (exposed to 50mg/100gm) with stunting in size, maldeveloped calvaria, malrotated limbs (arrow) and haemorrhage (red arrow) in head region on right is the control for comparison.

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