TERATOGENIC EFFECT OF ACE INHIBITOR (LISINOPRIL) AND CHRYSANTHEMUM INDICUM(GULDAUDI) ON HEAD AND FACE REGION OF MICE FETUSES

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ABSTRACT

Lisinopril an ACE inhibitor when administered to pregnant mice was found to be teratogenic. Chrysanthemum indicum extract was also administered to pregnant mice and compared with the teratogenic effect with those produced by lisinopril. Thirteen pregnant female mice were administered lisinopril orally in the dose of 2mg/100g and Guldaudi extract 50mg/100g body weight of mice. Lisinopril and Guldaudi were administered to pregnant mice daily from day zero of pregnancy. On day 18 mother mice was sacrificed and pups were collected. These showed significant stunting in size ($p<0.001$) in both lisinopril and guldaudi groups. Exencephaly was found in 26.08% in lisinopril group; however hypoplasia of skull was present in Guldaudi group. Resorption (5%) was noticed only in Guldaudi group. The lisinopril in dose of 2mg/100g body weight tried was found to be teratogenic and dose of Guldaudi extract in the dose of 50mg/100g was also found teratogenic with less severity as compared to 2 mg dose of lisinopril. Oligohydramnios and hypoxia following lisinopril and Guldaudi administration was attributed to be the cause of these malformations.

Key words: Teratogenicity, Lisinopril, Guldaudi, Mice.

INTRODUCTION:

The first ACE inhibitor was reported as a natural peptide isolated from a Brazilian snake's venom[1,2]. Using this peptide as a model system, specific ACE inhibitors such as captopril and enalapril have been synthesized and commercially used in the treatment of hypertension and prevention of chronic heart failure. However, these synthetic drugs have side effects such as cough, taste disturbances, and skin rash. Therefore, various plant products[3,4,5,6,7] that were generally recognized as safe were screened for ACE inhibitor activity. Compositae family plants have been used in oriental medicine[8]. To assess the efficacy of the Compositae plants for hypertension, thirty Compositae plants were screened for ACE inhibitory activity. Among them, Chrysanthemum boreale Makino had the higest ACE inhibitory activity. Each part of the plants was examined for ACE inhibitory activity and the flowers of the Chrysanthemum boreale Makino had the highest inhibitory activity[8].

This Chrysanthemum indicum species of Compositae family is also known to contain ace inhibitory activity in their flowers[8]; therefore it was decided to work on this topic to find out if this plant product also has antihypertensive property, as well as teratogenicity in mice and compared with lisinopril an ACE inhibitor.

Exposure to ACE inhibitors during the first trimester cannot be considered safe and should be avoided[9].The present work was conducted to see if plant extract from flower of Chrysanthemum indicum is teratogenic and in what respect the teratogenicity differs from ACE inhibitor Lisinopril.

In the early part of experiment many dose were tried. Some of these doses found totally fatal others i.e.1mg, 2mg, 5mg/100gm body weight of mice were found to be suitable for the experiment out of these three 2mg/100gm was the most suitable.

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. These were primigravida having an average age of 50 days and body weight ranging between 25- 45 grams. These mice were kept in separate individual cages in the animal house 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

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

RESULTS

On gross evaluation of head and face of lisinopril treated group, it was observed that it had conspicuous shape of the snout which was flat anteroposteriorly and wide side to side. Eye could not be seen on any of the two sides. The brain showed exencephaly and the calvaria was absent. Brain was covered with a membrane. Pinna was visible on left side only (fig-1).

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	1.3647	0.08324	0.01471	% less than average
Lisinopril 2mg/100gm	46	.60-1.68	1.0522	0.25034	0.03691	22.90%
Guldaudi Extract 50mg/100g	31	1.30-.90	1.1273	0.11261	0.03395	17.39%

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

As depicted in Table-1 the control group fetuses had an average weight of 1.3647 ± .08324g. In treated groups the average weight of fetuses of 2mg lisinopril group, was remarkably less. The average weight of guldaudi group fetuses was also less as compared to control but less than lisinopril group.

Control Group	Lisinopril Group	Guldaudi Group
233.75 µl	148.15 µl	154.84 µl

As depicted in Table-2 the control and treated groups 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 p<.001.

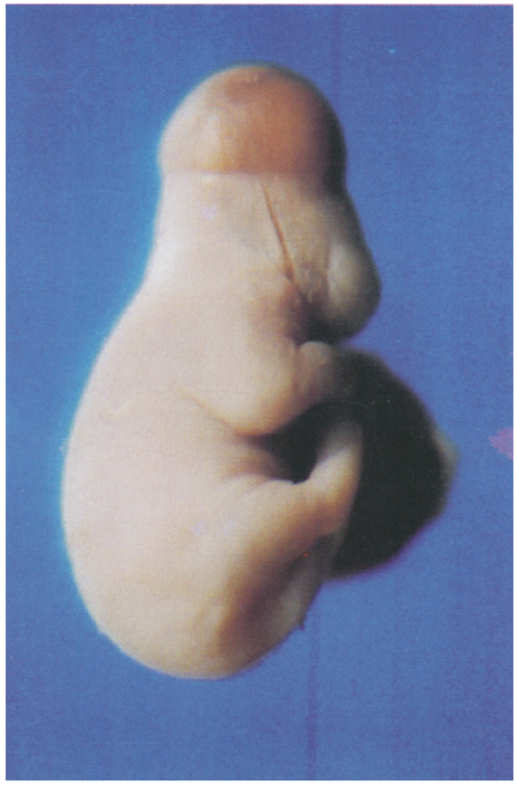

Fig-1 Right lateral view of 18 days old fetus from (2 mg lisinopril dose group) photograph shows head and snout malformations.

Fig-2 Eighteen day old fetus (left) treated with Guldaudi extract shows stunting in size and hypoplastic calvaria. On right side is the control fetus for comparison.

In Gross evaluation of Guldaudi treated pups stunting in size, maldeveloped calvaria were observed as seen in (fig -2). On the right is the control pup for comparison.

DISCUSSION:

The results described above suggest that ACE inhibitors are teratogenic. In the present study both lisinopril and extract of Guldaudi were administered

to pregnant mice throughout pregnancy to study their effect on the development of head and face. Administration of lisinopril is known to cause oligohydramnios[1] and flowers of Guldaudi are known to have to ACE inhibitor like substance.

It was anticipated that these two preparations will reach the fetus crossing the placental barrier and may interfere in the development of kidneys altering its structure and function. The damage would result in oligohydramnios causing pressure on fetus directly by the membranes and the uterine musculature.

Due to involvement of the kidney the amount of the urine excreted by fetus into the amniotic fluid /sac would be less in quantity causing oligohydramnios.[10]. Severity of oligohydramnios[11] would also be increased due to failure of addition of secretion from the lungs of the pups under going hypoplasia[12] due to ACE inhibitor action.

As seen in table-2, when studied in the control vs lisinopril/ treated pups there was significant reduction in the quantity of the amniotic fluid. The average quantity in the control sacs was 233.75 µl where as in lisinopril treated sacs it was reduced to 148.15 µl and in Guldaudi treated sacs it was 154.85 µl on day 18th of gestation ($p<.001$).

Oligohydramnios is known to cause limb malformation, exencephaly, hydrocephalus, anencephaly, spina bifida, cleft palate and many more malformation in rat[13].

This was one of the principle findings of the work for the reason that the ACE inhibitors cause renal and lung damage during development as a specific assault.

The oligohydramnios secondary to renal and lung damage itself is known to be highly teratogenic[14].

To conclude, in present study it was found that the fetus had conspicuous shape of the snout which is flat anteroposteriorly and wide side to side. Eyes were absent on both side. The brain showed exencephaly and the calvaria was absent. Brain was covered with a membrane. Pinna was not visible on right side in 2 mg lisinopril treated group (fig-1). In Gross evaluation of guldaudi treated pup had stunting in size, and hypoplasia of calvaria was found.

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