ANOMALIES INDUCED BY LOSARTAN, IN DEVELOPING MOUSE

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ABSTRACT: The present study was carried out to observe developmental defects induced in mice (*Musmusculus*) by losartan, an antihypertensive drug. Losartan was administered orally in four doses including 1.5, 2.0, 2.5 and 3.0 µg/gBW. It was administered on 8th day of gestation. Distilled water was used in control group. The fetuses were recovered on 18th day of gestation to observe morphological and histological changes. The morphological observations of fetuses showed anomalies like open eye lid, distorted body axis, kyphosis, skin hemorrhage, drooping wrist, microphthalmia, low set arm, kinky tail, exencephaly anophthalmia, maxillary macrognathia, microcephaly, clubbed foot and microtia. Morphometric observations showed that with increase in losartan concentrations there was reduction in body weight, brain circumference, eye circumference, limb size, tail length and crown rump length. Abnormalities found in histology were enlargement of 3rd and 4th ventricle, cleft palate, ventricular septal defect, cleft palate and dysplasia of kidney. It was concluded that losartan was toxic to developing fetus.

Key words: Antihypertensive drug, Losartan, developmental anomalies and mouse fetus.

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INTRODUCTION

Losartan is an angiotensin II receptor antagonist (Satoh *et al.*, 2016). It is used for the treatment of hypertension and other diseases such as ventricular hypertrophy, cerebrovascular accidents and diabetic nephropathy (Krupika *et al.,* 2008 and Kurokawa *et al.,* 2006)

Losartan has molecular specific effect. High dose losartan therapy is more efficacious in reducing proteinuria and preserving renal function (Woo *et al.,* 2009).In patients with diabetes 2 and nephropathy, losartan produces renal benefits (Brenner *et al.,* 2001). Upon oral administration losartan undergoes extensive metabolism in liver and is converted into 5 carboxylic acid metabolites. Peak plasma concentration of metabolite is achieved within 2-4 hrs post oral administration. The major metabolic pathway for losartan is by the cytochrome P450 (cyp) 3A4, 2C9 and 2C10 isozymes. Losartan has active metabolite that prolongs its duration of action (Burnier and Macllar, 2001).

Cytochrome P450 (cyp) 3A4, 2C9 and 2C10 Isozymes are metabolic pathways for losartan. Its use in stroke management improves endothelial function and vascular structure (Michiaki *et al*., 2012). It helps to minimize vascular oxidative stress, myocardial fibrosis and ventricular hypertrophy (Diez, 2006). Intranasal losartan and other angiotensin receptor blockers are used at concentration below the threshold. Losartan is used for the treatment of Alzheimer (Danielyan *et al.,* 2010), Blood pressure and incidence of serum creatinine in kidney disease (Carswell and Goa,2003 and Schrader *et al.*,2002)

There is a report of young woman who while receiving losartan treatment developed cough (Mutolo *et al* 2013). Losartan potassium is metabolized in liver so it may cause hyperkalemia (Wolfgang and Winkelmayer., 2015). During the treatment of hypertension by losartan, it induced hepatic injury which was seen during initial phase of treatment (Tabak *et al.,* 2002).

Angioedema developes in patients treated with losartan (Abdi *et al.,* 2002). Short term treatment of losartan slightly attenuates symptomatic and hormonal responses of hypoglycemia (Deininger *et al.,* 2001). Anhydroamnios are diagnosed at 31st week of gestation in 31 year old woman. Ultrasound confirms intrauterine fetal death. Limb and face are deformed in fetus. At autopsy hyperplastic skull bones with wide suture and pulmonary hypoplasia are seen (Saji *et al.,* 2001).

The Present study was conducted to observe the effects of losartan on development of fetuses during gestation. The result may help in use of losartan carefully, if drug appear teratogenic.

MATERIAL AND METHOD

The 40 pregnant mice were placed in four experimental groups with 10 animals in each. Experimental groups were further subdivided into four subgroups single, double, triple and chronic exposure groups. Different concentrations of losartan were made through dilutions in water, in such way that 0.1 ml of the solution contained desired amount of drug .The experimental animals belonging to groups I, II, III and IV were orally administered with doses of 1.5, 2.0, 2.5 and 3.0µg/g BW,

respectively on 8th day of gestation. The animals in control group were given 0.1 ml of distilled water as vehicle control.

Fetuses were recovered on day 18 of gestation. Abnormalities were carefully observed. Selected fetuses with morphological anomalies were macro photographed and compared with control.

For abdominal incision on day 18 of gestation pregnant females were weighed and anesthetized using anesthetic ether. The uteri were weighed by using digital balance and photographed in-situ for the purpose of gross morphological changes. The implantation sites in gravid uteri were carefully observed and recorded to count number of resorbed, dead, live and malformed fetuses. Finally implanted fetuses with intact amniotic membranes and placentas were obtained.

Fetuses removed from the uteri were carefully weighed and observed. Stereoscopic binocular microscope was used to find external abnormalities. Fixation was done in Bouin's fixative for 48 hours at room temperature and fetuses were transferred to 70% ethanol. (Carson, 1992 and Patki *et al*., 1992).

The morphological studies comprised of careful observations of the craniofacial, trunk, tail, limb and axis of fetuses. Morphological defects in fetuses were counted and tabulated. After morphological study fetuses were carefully selected for macrophotography. Measurement of fetal body weight, eye circumference, head circumference, length of snout, forelimb, hind limb, tail axis of body and CR length were recorded.

The head circumference value (P = mm^2) for each fetus was measured using: "Ellipse circumference calculator" a computer based program (CSGN, 2006). Head circumference, measurements of fetal occipito-frontal (AB) and width bi-parietal distance (CD) was calculated as.

Pre-calibrated ocular micrometer and stereoscopic binocular microscope labomed were used for the measurement of fetal eyes and other parameters.

The values were statistically analyzed by ANOVA on the software SPSS followed by Duncan's Multiple Range Test (DMRT) for multiple comparisons of each dose group.

RESULTS AND DISCUSSION

In group I, fetuses were with abnormalities such as kyphosis (2.08%), drooping wrist (2.08%) and kinky tail (1.04%). In case of group II, anomalies observed were skin hemorrhage (2.5%) imbalanced body axis (5.0%), microphthalmia (2.5%), degenerated claws (3.75%) and kinky tail (3.75%). In group III, abnormalities in fetuses were exencephaly, anophthalmia, maxillary macrognathia, sacral hygroma (7.4%), micromelia (5.5%), degenerated claw(5.5%).In IV group anomalies present were exencephaly, anophthalmia, low set arm, clubfoot, microtia. microcephaly (4.5%), open eye lid (4.5%), hemorrhagic spot (6.81%).In control group few abnormalities like syndactyly (0.91%) and hemorrhagic spots (0.91%) are present (Table1, Fig 1).

Table 1: Morphological anomalies in 18 days old mice fetuses recovered from mothers treated with different doses of losartan on day 8th of gestation.

Groups	No. of Fetuses	Axis (%age)	Brain (%age)	Eye (%age)	Limb (%age)	Claws (%age)	Hemorrhagic Spots (%age)	Tail (%age)
0.0	110	0.00	0.00	0.00	0.00	Syndactyly (0.91)	Hemorrhagic spots (0.91)	0.00
1.5	96	Kyphosis (2.08)	Hydrocephaly (3.0)	0.00	Drooping wrist (2.08)	0.00	0.00	Kinky tail (1.04)
2.0	80	Imbalanced axis (5.0)	0.00	Microphthalmia (2.5)	0.00	Degenerated claws (3.75)	Hemorrhagic spots (2.5)	Kinky tail (3.75)
2.5	54	Sacral hygroma (7.4)	Microcephaly (4.5)	0.00	Micromelia (5.5)	Degenerated claws (5.5)	0.00	0.00
3.0	44	Kyphosis (6.81)	Exencephaly (4.5)	Open eyelid (4.5)	0.00	0.00	Hemorrhagic spots(6.81)	0.00

Table 2: Toxicity of losartan potassium on pregnant mothers and developing fetuses, exposed to different doses on 8th day of gestation.

Dose groups	females treated (N)	Total no. of fetuses recovered	malformed fetuses (%)	resorbed fetuses (%)
0.0	10	110	1.82	0.00
1.5	10	96	3.13	2.08

2.0	10	80	15.0	2.5
2.5	10	54	18.52	3.7
3.0	10	44	22.73	6.82

N: number of female treated

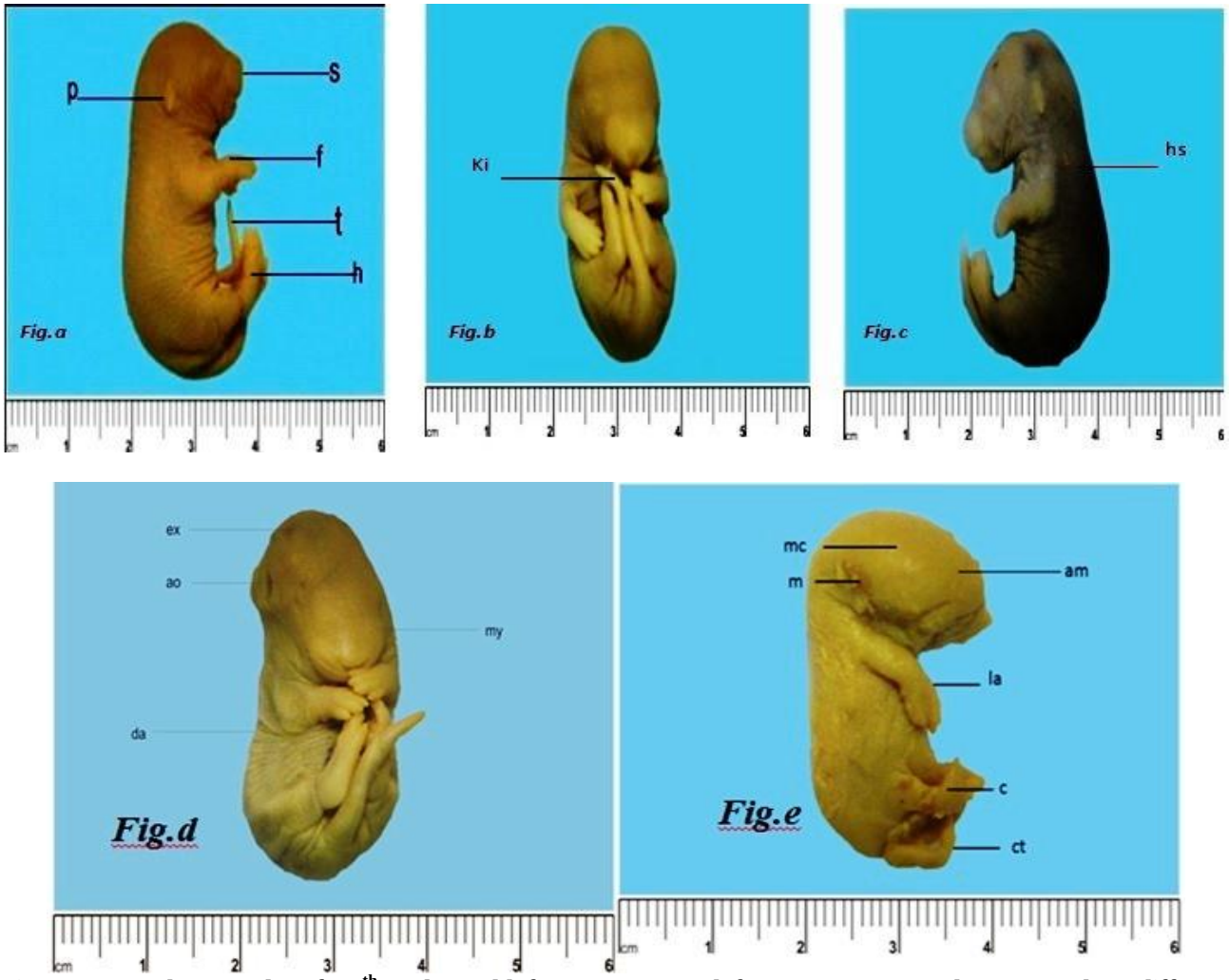

Fig 1. Macrophotographs of 18th days old fetuses recovered from pregnant mother exposed to different concentrations of Losartan.a:0.0µg/g, b:1.5 µg/g, c:2.0 µg/g, d: 2.5µg/g, e: 3.0 µg/g.

Note: snout (s);pinna (p);forelimb(f);hind limb (h) tail(t); Kinkytail(ki), Skin hemorrhage(hs), exencephaly (ex); anophthalmia (ao); distorted body axis (d);maxillary macrognathia(my), microcephaly (mc); low set arm (la); clubbed foot (c); microtia(m) and curly tail(ct)

Histological Lesions

Brain region: In dose group I (1.5µg/g BW), III (2.5µg/g BW) and IV (3.0µg/g BW) enlargement of 3rd and 4th ventricles was observed. There was presence of undifferentiated ectoneuralcells. Herniation of 3rd and 4th ventricles was found (Fig. 2a, b and c).

Eye, nasal and tongue region: In dose group III (2.5µg/g B) cleft palate and poorly developed pharynx were present (Fig.2a and c).

Cardiac and pulmonary region: In dose group III (2.5µg/g BW) pulmonary hypertrophy and congenital adenomatoid malformations were present (Fig.2d). In group II (2.0µg/g BW) there was exposure of heart. In group III (2.5µg/g BW) and group IV (3.0µg/g BW dose groups) ventricular septal defect and enlarged ventricular chambers were observed (Fig.2f). There was also agenesis of heart muscles.

Kidney region: Abnormalities of kidney region in group I (1.5µ g/g B W) and group II (2.0µ g/g B W) dose groups were dysplasia of kidney and degeneration of muscle fiber (Fig. 2e).

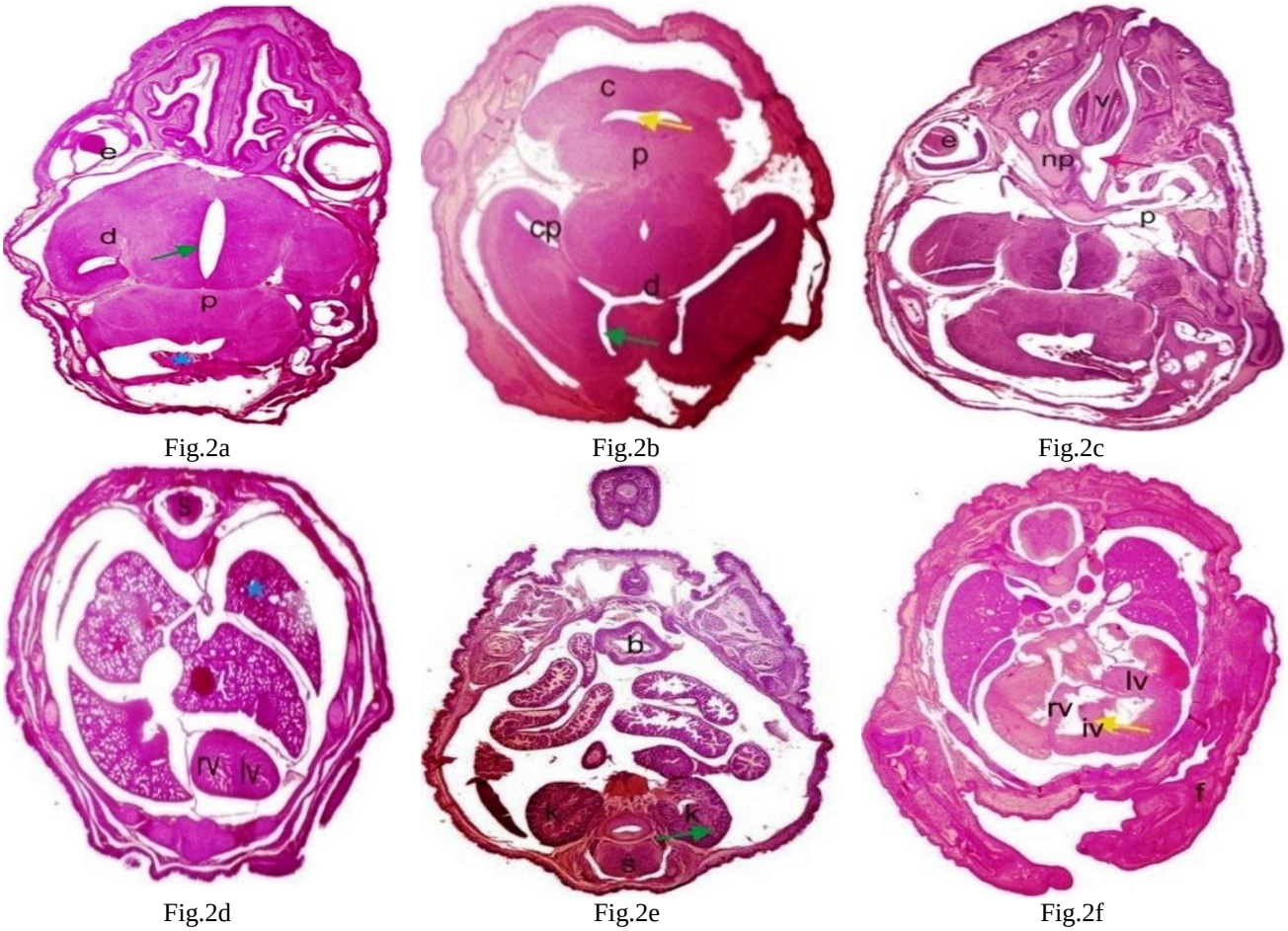

Fig.2a Fig.2b Fig.2c

Fig.2d Fig.2e Fig.2f

Fig 2 (a, b, c, d, e and f): Histological lesions observed in different organs of treated mice through a: eye, b: brain, c: pharyngeal, d: pulmonary, e: kidney region, f:cardiac

e: eye; c: cerebral hemisphere. d: diencephalon: pons; n: nasal pouches; yellow arrow: herniated 4th ventricle; dark green arrow: herniated 3rd ventricle, blue star: undifferentiated ectoneural cells; vc; vomerocentral organ; pink arrow: cleft palate; hollow star: ectoneural cells. ph: pharynx; s: spinal cord; ln: lung ; black star: Plumonary hypertrophy; pink star: congenital cystic adenomatoid malformation. h: heart; rv: right ventricle; lv: left ventricle; f: forelimb; black arrow: ventricular septal defect; k: kidney ; b: bladder; blue arrow: dysplasia of kidney.

Between vehicle control and all the dose groups a significant increase in ($p<0.001$) percentage of malformed fetuses was observed (Table II).While, a significant increase ($p<0.001$) in percentage of resorptions was observed between vehicle control and dose groups 1.5,2.0,2.5 and 3.0 µg/g BW (Table II).

DISCUSSION

Losartan was highly selective and specific in its action without any adverse effect. The aim of using antihypertensive treatment was to reduce cardiovascular and cerebrovascular events with high blood pressure. In a double blind heart failure endpoint evaluation it was found that 150mg of losartan had favourable effect compared with currently recommended 50mg per day (Doehner *et al*., 2010).

There are few reports of losartan toxicity reported by Nayar *et al*, (2003) who found a case of incomplete ossification of skull bones, transient oliguria and feed intolerance in a newborn, following in utero exposure to losartan. Therefore, present study was carried out to draw conclusion about teratogenic effects of losartan during pregnancy.

Morphological anomalies found in the present study were in the head region such as microcephaly, anencephaly, exencephaly, hydrocephaly. Brass and Faix (2006) studied a case of losartan toxicity in 41 years old woman. Ultrasound examination of fetus two days prior to delivery revealed anhydroamnios and empty bladder. Other anomalies found in fetus were oliguria, hyperkalemia, renal dysfunction, respiratory failure and joint contractures. In the present research work more pronounced effect of losartan was fetal and maternal

weight reduction. There was reduction in litter size too. It was observed that there was an increase in rate of malformations and resorptions with an increase in concentrations in different dose groups and subgroups.

In a study Sanchez *et al.,* (2009) stated similar results. They viewed that after administration, losartan binding with cerebellar nuclei was increased due to blockage of AT (2) receptor. This blockage seemed to arrest cerebellar cortex development. Von *et al.,* (2008) concluded that losartan administration during middle and late pregnancy, affected both at histological and receptor localization levels.

In a double blind heart failure endpoint evaluation it was found that 150mg of losartan had favourable effect compared with currently recommended 50mg per day (Doehner *et al*, 2010). In this way, 150mg prevented one primary event for 31 patients treated for 4 years. According to Bakkum *et al.,* (2006) losartan produced oligohydroamnios at 27 week of gestation in human. Limb anomalies found were amelia, micromelia, malrotation of limbs, low set arm, hyper flexion and drooping wrist. In digits, syndactyly and degenerated claws were found and clubbed feet. Saji *et al* (2001), reported anhydroamnios, facial and limb deformities in fetus at 31 week of gestation. Ultrasound confirmed intrauterine fetal death. Autopsy examination revealed pulmonary hypoplasia and hypoplastic skull bones with wide sutures.

Cardiac defects found were dilation of ventricles, myocardial degeneration (Fig.11), reduced ventricular development and cardiac dilation as compared to controls.

In the current study kidney anomalies were dysplasia of kidney and degeneration of muscle cells in group I and II. According to Sanchez *et al.,* (2008) kidney organogenesis was affected by losartan (1mg/Kg/day) administration to one week old fetuses. Enlargement of Bowman spaces and alternation in glomeruli structure was found. Snout abnormalities found were micrognathia, maxillary macrognathia and agnathia.

Conclusion and Recommendations: it was concluded that losartan is potentially teratogenic to developing fetus of mice losartan .Since mice is a mammalian model therefore losartan is also suspected to be teratogenic in human beings and has toxic effects on fetal organ like liver, brain, kidney, lungs and skeleton Therefore its use should be avoided during pregnancy. If its use is necessary then precautions be taken.

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