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# 'Stage specific teratogenic effects of Retinoic Acid on Mouse embryos'.

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## ABSTRACT

Retinoids are well known agents which causes teratogenesis in vertebrates. To understand whether Retinoic Acid (RA), a potent form of retinoids, causes teratogenesis by influencing organosis of all the three primary embryonic layers in a uniform pattern or in different manner, pregnant swiss albino mice were orally given single dose suspension of Retinoic Acid (RA) in 200µl dimethyl sulphoxide (DMSO) on 5$^{th}$ 8$^{th}$, 11$^{th}$, 14$^{th}$ and 17$^{th}$ day gestation. Control group of mice were administredorally only 200µl of DMSO on the same gestation periods. The different sensitivity response of heart,skin and liver was studies under this treatment. These organs develop at different gestation periods from different primary embryonic germ alyers. The present study indicates differential sensitivity response of heart , liver and skin to retinoic acid. Heart shows maximum teratogenesis by RA on 11$^{th}$ day gestation, liver showed on 8$^{th}$ day and skin on 14$^{th}$ day gestation periods.

**Key Words**: Retinoic Acid, Mouse embryo, Development,Teratogenesis

## INTRODUCTION

Vitamin A is required for normal growth, reproduction, vision, maintenance of normal skin and epithelia of respiratory, urinary, gastrointestinal tracts and process of differentiation during development in vertebrates (Moore, 1967; Hinchliffe and Johnson, 1980; Johnson and Scadding, 1991).

Retinoids are teratogenic agent, when administered exogenously, they cause a variety of defects of morphogenesis and embryogenesis during development in vertebrates. (Kochhar, 1967; Tuli, 1968, Deluca, 1975; Summerbell, 1983; Deluca, et al., 1996).

Administration of excess retinoic acid in mammalian abnormalities pregnant female produce a variety of defects and inner in the fetus including cleft palate, shorting of jaws inner ear, central nervous system, shorting or absence of limb(Cholan,1953,1954; Girud,1954; Kalter and Warkany,1959,61;Takekoshi,S.,1964;Langman,et.al.1966;Lorente,et.al.1976' Sulik and Dehart,1980).Recently, retinoids have been found to influence development of other organs such as heart, urinary system skin,liver, nervous system, limb and skeleton (Ong, et al1976, Sani et al. 1974; ; Goodman, 1996). Dickman, et al., 1996; Elmazar, et. al., 1996; Goodman,1996).

## MATERIAL AND METHODS

Randomly bred swiss albino mice were used in the present study. They were maintained under controlled light and temperature conditions and were fed balance food pellets (Brooke Bond Lipton India Ltd.), supplemented with green vegetables and germinated seeds. Six to Nine week old females were housed in mating cages in the ratio of four female and one male. Every morning females were observed for the presence of vaginal plugs. The day on which vaginal plug and sperm in the smear preparation were detected was considered as day zero of gestation.

Such pregnant females were divided into six groups according to the gestation periods as Group A (5th day gestation), Group B (8th day Gestation), group C (11th day gestation), group D (14th day gestation) and Group E (17th day gestation). To the pregnant females of each group a single dose of RA (4mg Retinoic Acid (Sigma, London Chemicals) was given in suspension of 200µl Di Methyl Sulphoxide (DMSO) (B.D.H. chemicals Pools, England). of Every experimental group was run with a control group. Females of groups were given equivalent volume of control Dimethyl Sulphoxide. The animal of each group were anaesthetized with ether on 19th day gestation and their embryos were removed for observations. Embryos were fixed in Bouin's fluid and preserved in 70% alcohol. Selected cases were photographed and others processed for intoto staining or histological observations. Were processed for intoto stating or histological observations.

## RESULT

Morphological observation based on external fetures of the respective organ and tissue assess (heart, skin and liver ) organisation of these organs. Were the main criteria to normal pattern of development and deviation from the normal pattern as a result of teratogenesis.

To quantify the extent of teratogenecity arbitrary unit indicating percent of sensitivity were designated and skin, liver, heart. generally showed this type of observation.

Heart

In the case of heart, the earlier symtoms of teratogenecity was recorded disruption of pericardial membrane and the teratogenesity considered in terms of 20 arbitrary unit (AU) .In such case there were patches of disrupted vascularisation. In more severe case there was curvature of ventricles (40 AU). In the highest teratogenecity of heart was observed in terms of bulging of left ventricles (60 AU). The normal pattern of Heart development range from 0 to 10 AU. The cases with extreme teratogenecity which did not survive designated between 80 to 100 AU. was

Skin

The quantification of teratogenecity in skin was observed as folding of skin (20AU), occasional blood patches throughout the body embryo (40 AU) disruption and shrinkage of skin(60AU). In extereme condition of teratogenecity, skin was severely damaged having wide blood patches and wounded (80 to 100AU) . Such cases did not survive.

LIVER

Teratogenic effect of RA were also observed on liver, where the earlier symptom of teratogenicity were recorded as separation of lobes (20 AU). In more severe cases there was not only increase in gap between lobes but also breaking in upper lob areas. There were wounded areas of the lobes in such cases (40 AU). Liver lobes were swollen with occasional blood spots and further separation between the lobes (60 AU). In the extreme cases of liver defects the lobes were badly disorganized, swollen, having broken spots and blood patches (80 to 100 AU.)

The above levels of teratogenecity were observed different at different gestation periods as has been shown by the bar diagram. These graphical presentations (Fig. 1, 2 and 3) clearly indicates that the teratogenic effects produced by RA are stage dependent and the sensitivity of liver is to RA is maximum at 8 dpg, skin is at 14th dpg and heart is 11th dpg.

## DISCUSSION

Vitamin A is known to cause teratogenesis during vertebrate development. The development of heart is endomesodermal, skin is ectomesodermal and of liver endodermal (Developmental biology, Scott F. Gilbert, 1988). These primary germ layer are different state of differentiation during organogenesis of various organs. Therefore the effect of vitamin A is not only at specific to the ectoderm, mesoderm and endoderm but also dependent on the specific stage of development of these germ layers. Stage dependent effect of retinoids has been confirmed by many investigators on limb development chick embryos (Tickle,et al.1982, and 1982 Summerbell, et al.1983) and regeneration of limbs in amphibian (Niazi and stocum, 1985; Niazi et al., 1989). Very little is known about how vitamin A would influence development of heart, Skin and Liver which are having component from ectoderm, mesoderm and endoderm in specific organization.

The results of present study clearly demonstrate differential sensitivity of these organ to RA. Liver morphogenesis was influenced most, when treatment was on 8th day gestation, where heart development showed highest rank of teratogenecity of living embryos (60 AU) on $11^{th}$ gestation. Although, skin differentiation was affected in the 8th and 11th day treated groups but the most severe effects of RA were observed on 14th day gestation. RA has been found to induce teratogenic effects in heart,skin and liver by many investigator (Fisher, et al., 1996;Leelaprute, et al., 1973; Dickman and smith 1996 ) To understand differential sensitivity of the three organs of RA, it is required to understand different state of differentiation of three primary germ layer during organogenesis of these organ using parameter of biochemistry and ultra structure study.

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gs. 1-3 : Teratogenic effects of 4 mg RA on development of Heart (fig.1), Skin (fig.2) and Liver (fig.3 ) .AU. = Extent of teratogenecity in Arbitrary Units ; dpg = days post gestation .