International Journal of Scientific & Engineering Research, Volume 6, Issue 3, March-2015
ISSN 2229-5518

Evaluation of Teratogenic potentials of Bronchodilator drug on offsprings of Albino rats

Abd El Wahab El Ghareeb, Hamida Hamdi, EL-Sayed Fahim Taha, Heba Ali

Abstract— The present study aimed to evaluate the teratogenic effects of the mucolytic and broncholytic drug (mucophylline) administered daily orally to the pregnant rats and nursing rats. The pregnant animals treated during the gestational period (5th – 18th day of gestation) with doses 30.83 mg/Kg and 66.61 mg/Kg the human equivalent dose (HED). On the 19th of gestation, the animals were sacrificed and the numbers of implanation sites, resorbed and live fetuses were counted. The fetal weight, length and tail length were recorded. Results showed decreased weight gain, fetal growth retardation during gestation period was dose dependent. Hematoma and anomalies of limbs were detected morphologically in the fetuses of maternally treated groups. Fetal skeletal abnormalities included lack of bones ossification as well as unossified centers of cervical, thoracic and sacral vertebrae and dumbelled shape vertebrae and bent ribs.

Key words — Teratogenicity, Pregnant rats, Skeletal malformation, Bronchodilatordrugs.

1 INTRODUCTION

ABOUT 1% of pregnant women suffer from active asthma (Schaefer and Silverman, 1961, Gordon, 1970 and De Swiet, 1977). Current evidence suggests that uncontrolled asthma may lead to maternal hypoxemia with potentially grave consequences for the fetus. Asthma in pregnancy should therefore be treated promptly and appropriately to reduce perinatal mortality and morbidity. As in non-obstetric practice, management of pregnant women with asthma aims at preventing recurrent attacks of wheezing, status asthmaticus, and respiratory failure. Drugs that are teratogenic or detrimental to the fetus should obviously be avoided. The main classes of antiasthmatic drugs are: (a) bronchodilators, such as β adrenergic agonists and theophyllines, and (b) prophylactic or anti-inflammatory agents such as corticosteroids (oral or inhaled) and sodium cromoglycate.

The dam and fetus have a close relationship with each other via the placenta and form the maternal-fetal-placental unit in mammalian embryonic development. Drug- or chemical-induced placental functional depression and injury subsequently result in abnormal fetal growth or development leading to fetal resorption or teratogenicity.

Effects of Antiasthmastics (Bronchodilators); Xanthines: The group of substances referred to as methylxanthines include caffeine, theophylline, theobromine and aminophylline, which is a compound of theophylline with ethylenediamine. They are mainly absorbed from coffee, tea and cocoa products such as cola beverages and chocolate bars, as well as some medications. Theophylline is an alkaloid found in tea (Thea sinensis) and chocolate and is structurally related to caffeine and theobromine. Theophylline is used as a pharmaceutical agent.

Theophylline was administered continuously in the feed applying the dosages 0, 124, 218, and 259 mg/kg bw/d in Sprague-Dawley (CD) rats. The incidence of litters with one or more external, skeletal, or visceral malformations was also unaffected by theophylline treatment. No teratogenitic effect at any of the doses was seen (Lindstroem et al., 1990).

Animal studies (with oral exposure to theophylline ranging from 124-500 mg/kg bw/day), showed reductions in the number of pups per litter in mice (Lamb et al., 1997 and Morrissey et al., 1988) and rats (Lindstroem et al., 1990), increased percentage of resorptions in mice 10 and reduced pup weights in mice (Lamb et al., 1997, Lindstroem et al., 1990 and Morrissey et al., 1988) and rats (Lindstroem et al., 1990). Some of these effects were noted in the absence of maternal growth retardation. In these studies, the administration of theophylline did not induce visceral or skeletal malformations and variations.

Mucophylline syrup (mucolytic and bronchodilator drug): Composition: Each teaspoonful (5ml) contains: Bromhexine hydrochloride 4mg and Acephylline piperazine 100mg. Chemical name: Piperazine 7-theophyllineacetat.

It acts on the bronchial tree clearing it from the viscid sputum and relaxing its constricted muscles providing an efficient symptromatic treatment in most respiratory ailments through the action of its two active ingredients. Bromhexine hydrochloride is a mucolytic agent which acts by depolymerization of the mucopolysaccharide ground substance of the sputum increasing its fluidity so facilitates its expectoration and minimizes the intensity and frequency of cough. Acephylline piperazine is a theophylline derivative with a direct bronchodilator action. Its safety during pregnancy is not established and use in caution in nursing mothers. FDA Pharmaceutical Pregnancy Category (N) for Acefylline Piperazine and Bromhexine Hydrochloride is not available for Risk Classification. This study will add to the medical literature on Mucophylline syrup exposure during pregnancy and will provide insight to women and healthcare professionals about the risks to the fetus.

2 MATERIALS AND METHODS

2.1 Experimental animals

The present experimental study is carried out on the albino rat (Rattus norvegicus). The standard guidelines of the Institutional Animal Care and Use Committee (IACUC) were used in handling animals.

Females of 11-13 weeks old were selected for the present study and viginal smears prepared every morning and examined

International Journal of Scientific & Engineering Research Volume 6, Issue 3, March-2015
ISSN 2229-5518

under the light microscope according to the method of Snell (1956) for 5 days to select those in the pro-estrus. Two females with regular estrus cycle were selected in the pro-estrus stage and caged together with one male over night under controlled environmental conditions of temperature, humidity and light. The first day of gestation was determined by the presence of sperms in the vaginal smear (McClain and Becker, 1975).
A daily record of the weight of the pregnant females was made throughout the whole gestational period. The percentages of abortion were calculated in each group; abortion was determined by the presence of blood drops and sudden drop in the weight of the pregnant females.

2.2 Experimental strategy

Drug: The mucophylline was purchased from Misr Company for pharmaceutical industries, S.A.E., Egypt.
Experiment (Gestation group)
Duration of drug administration: 14 days, from the 5thday, day after day during gestation.
The animals were divided into three groups with twenty animals in each.
Group A: Control rats received distilled water orally.
Group B: Rats treated with 30.83 mg/kg of body weight of mucophylline administered orally.
Group C: Rats treated with 61.66 mg/kg of body weight of mucophylline administered orally which is (HED).

2.3 Developmental observations

On the 19th day of gestation, all pregnant rats of groups (A-C) were sacrificed and total implantation sites, fetal mortality rate (resorped or still birth) and living fetuses were recorded. Fetal body weight, body length, tail length and external malformation were recorded. Head, neck and limbs were examined.

2.4 Skeletal examination

Fetuses were preserved in 95% ethyl alcohol and were stained with double staining of fetal skeletons for cartilage (Alcian blue) and bone (Alizarin red) according to the method described by Peters (1977).

2.5 Statistical analysis

All the values were presented as means (μ) ± standard errors of the means (S.E.M.) Comparison between more than two different groups was carried out using the one-way analysis of variance (ANOVA) followed by Turkey-Kramer's multiple comparison test (Armitage and Berry, 1987), where $P<0.05$ was considered significant. GraphPad Software InStat (version 2) was used to carry out the statistical tests.

3 Results

3.1 Morphological studies

Pregnant rat's toxicity:
External symptoms and mortality:
The pregnant rats treated orally with 30.83 mg/Kg (group B) and 61.66 mg/Kg (group C) of mucophylline during gestation (5th - 18th) showed no external signs of toxicity. No mortality cases were recorded; all the treated dams were survived to the study end.

3.2 Change in body weight gain

The maternal body weight was followed all over the period of gestation for the control and experimental groups.
The average maternal body weight was recorded for all groups on the 5th and the 18th day of gestation (Table 1& Fig. 1). There was a reduction in maternal weight gain in two treated groups (B & C) when compared with the control group (no significant difference across groups $P \geq 0.05$).

Table 1: Showing effect of mucophylline on fetus weight, fetus length, tail length, placenta weight and mother weight gain at 18th day of gestation.

Group	Fetus weight (F.WT)	Fetus length (F.L)	Tail length (T.L)	Placenta weight (P.WT)	Mother weight gain (M.WT)
Control (A)	53.42±9.43	0.56± 0.007	1.38± 0.01	5.68± 0.03	3.82± 0.07
30.83 mg/Kg (B)	42.19±4.56	0.53± 0.016	1.19±0.01	5.41± 0.09a,b	3.32± 0.19a,b
61.66 mg/Kg (C)	46.56± 7.39	0.52± 0.01a	1.08± 0.03a,b	5.04± 0.05a,b	2.71± 0.07a,b

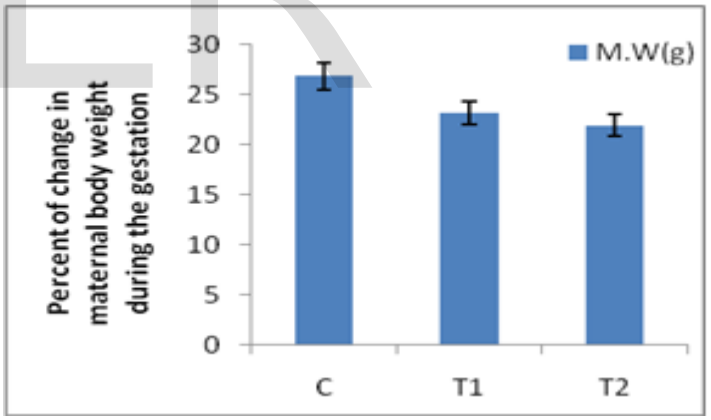


Fig. 1. Histogram showing effect of mucophylline on mother weight gain (M.W) at 18th day of gestation. Values are expressed as Mean ± SEM. The statistical differences were analyzed by ANOVA followed by independent samples T test. a= $P \leq 0.05$ compared with control and b= $P \leq 0.05$ compared with other treated group.

3.3 Morphological observations of uterus

The control uterus obtained from pregnant rats on day 18 of gestation showed normal distributed of the implanted fetuses between the two horns (Fig. 2). The uterus of pregnant rats treated with 30.83 mg/Kg showed asymmetrical distribution of fetuses in the two uteri horns and reduced number of fetuses (Fig. 3A), uterine horns showing clearly visible embryonic

International Journal of Scientific & Engineering Research Volume 6, Issue 3, March-2015
ISSN 2229-5518

resorped sites (Fig. 3B&C). The uterus of animals subjected to 61.66 mg/Kg showed asymmetrical distribution of fetuses in the two uteri horns (Fig. 4A&B) and Uterine horns showed resorption site and diminution in the number of implanted fetuses (Fig. 4C). Pregnant rats treated with low dose of mucophylline (30.83 mg/Kg) and high dose (61.66 mg/Kg) showed a significant increase in post-implantation loss index after comparison with the control group (Table 2& Fig. 5). While the animals from group B showed higher increase in post-implantation loss index than rats of group C, also there was significant difference across treated groups.

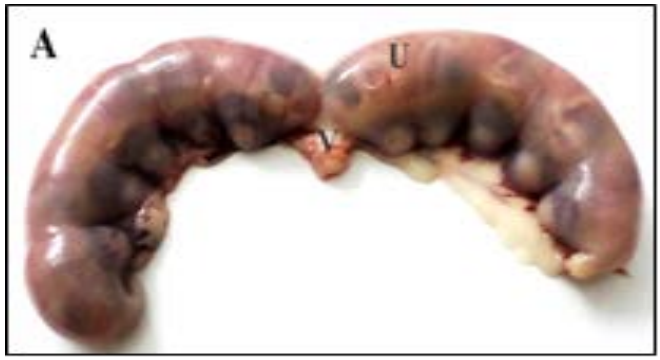


Fig. 2. A photograph of uterus of control pregnant rat at the 18th day of gestation; A) Showing normal symmetrical distribution of fetuses in the two uteri horns. U= Uterus, V= Vagina.

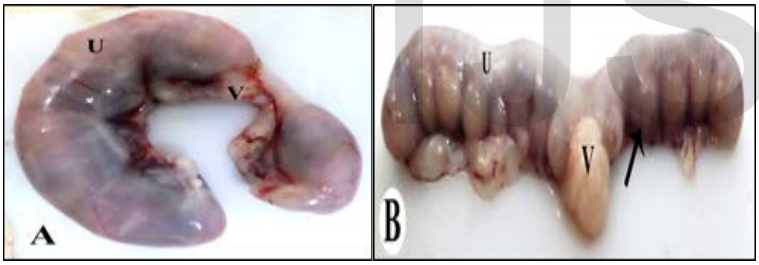


Fig. 3. Photographs of uterus of pregnant rat treated with 30.83 mg/Kg of mucophylline at the 18th day of gestation. Showing: A) Asymmetrical distribution of fetuses in the two uteri horns. B) Uterine horns showing clearly visible embryonic resorption sites (arrows).

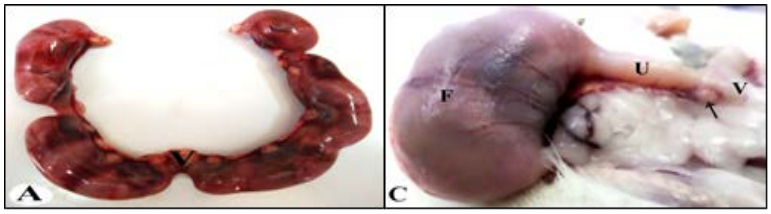


Fig. 4. Photographs of uterus of pregnant rat treated with 61.66 mg/Kg of mucophylline at the 18th day of gestation. Showing: A) Asymmetrical distribution of fetuses in the two uteri horns. C) Uterine horns showed resorption site (arrows) and diminution in the number of implanted fetuses.

3.4 Effect of mucophylline on fetuses

Growth retardation:

The morphological examination of the fetuses showed that the mucophylline caused growth retardation represented by decrease in fetal body weight, body length and tail length (Table 3& Fig. 6&8). There were significant (P≤ 0.05) reduction in fetus weight, fetus length and tail length of animals from (group B) and that from (group C) when compared with the control group (A), also there was significant difference across treated groups.

Table 2. Showing pregnancy outcome of rats treated with mucophylline. Values are expressed as Mean ± SEM.

Groups	Total no. of pregnant rats	No. of litter with viable fetuses	No. of litter with resorption	No. of litter with complete desorption	Implants / litter	Post-implantation loss index %
Control (A)	18	16	2	0	8.44 ± 0.14	1.19 ± 0.02
Treated Groups (5^{th}-18^{th}) 30.83 mg/Kg (B)	20	15	10	5	7.4 ± 0.015a, b	33.7 ± 0.14a,b
61.66 mg/Kg (C)	21	17	4	0	7.8 ± 0.013a,b	4.27 ± 0.11a.b

The statistical differences were analyzed by ANOVA followed by independent samples T test. a= P≤ 0.05 compared with control and b= P ≤ 0.05 compared with other treated group.

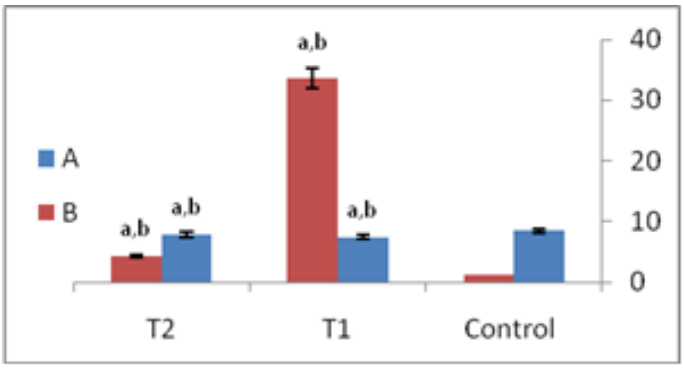


Fig. 5. Histogram showing number of implantation sites (A) and percentage of post implantation loss index (B) at 18th day of gestation after oral administration of pregnant rats with different doses of mucophylline.

International Journal of Scientific & Engineering Research Volume 6, Issue 3, March-2015
ISSN 2229-5518

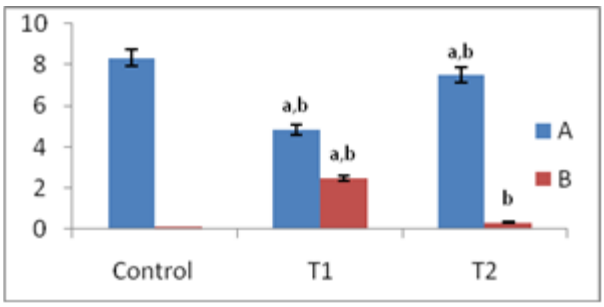


Fig. 6. Histogram showing number of live fetuses (A) and number of resorbed fetuses (B) at 18th day of gestation after oral administration of pregnant rats with different doses of mucophylline.

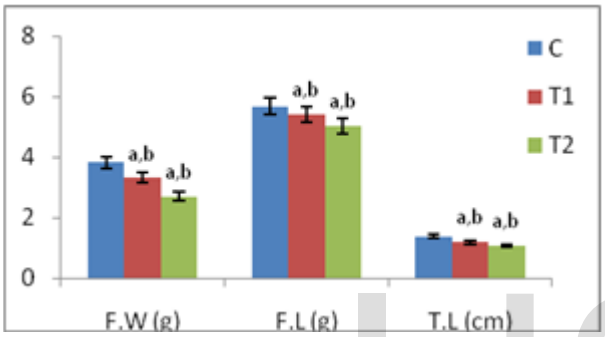


Fig. 7. Histogram showing effect of mucophylline on fetus weight (F.W), fetus length (F.L) and tail length (T.L) at 18th day of gestation.

3.5 Number of alive, desorbed and dead fetuses

There was a significant decrease ($p \leq 0.05$) in number of a live fetus of the pregnant rats that treated with 30.83 mg/Kg of mucophylline when compared with the control group. Treatment with HED of mucophylline caused a significant decrease ($p \leq 0.05$) in the number of live fetuses when compared with the control group. While the average number of live fetuses of group B was less than that one from group C.

Also, there was a significant difference in number of live fetuses ($p \leq 0.05$) between two treated groups (Table 3 & Fig. 8).

Fetuses maternally treated with low dose of mucophylline (30.83 mg/Kg) showed a significant increase ($p \leq 0.05$) in the number of resorbed fetuses compared to the corresponding control fetuses. Treatment with high dose of mucophylline (61.66 mg/Kg) caused no significant increase in the number of resorbed fetuses. There was a significant difference in number of resorbed fetuses ($p \leq 0.05$) between two treated groups (Table 3& Fig. 8).

There were no any cases of dead fetuses recorded at the two treated groups (Table 3).

Table 3. Showing incidence of resorption and live fetuses in treated rats at 18th day of gestation.

Groups	No. of alive fetuses/ litter	No. of desorbed fetuses/ litter	No. of dead fetuses/ litter
Control (A)	8.33± 0.18	0.11± 0.07	0
Group (5th-18th) 30.83 mg/Kg (B)	4.85± 0.821a,b	2.5± 0. 87a,b	0
61.66 mg/Kg (C)	7.47± 0.14a,b	0.33± 0.1b	0

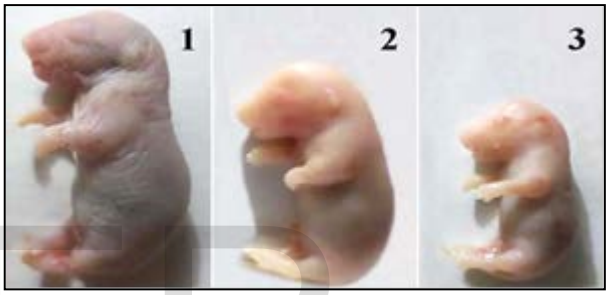


Fig. 8. A Photograph of fetuses at 18th day of gestation showing decreasing in weight.

3.6 Morphological malformations

The percentage of gross pathology of fetuses per dam was represented in Table (4) & Fig. (9). The most observed anomalies were, hematoma (red patches at different parts of bodies), fore and hind limbs malformations, microcephaly, exencephaly, short snout and kinky tail.

The fetus from control animals appeared with normal shape, correct weight and length (Fig. 10A&B), also showed (0.27± 0.1) hind limbs deformation and (0.72± 0.22) hematoma. Fetus of mother treated with 30.83 mg/Kg showed numbers of morphological anomalies such as, (0.65± 0.19) hind limbs deformation (Fig. 10) ,while there are no fore limbs deformation, (0.25± 0.09) short snout , (0.05± 0.05) microcephaly, (0.1± 0.06) deformed tail and the percentage of hematoma was significantly less than that of control fetus. The fetus of rats from group C (61.66 mg/Kg) showed incidence of gross pathological observations represented in, (1.4± 0.22) hind limbs deformation, there are (0.71± 0.15) fore limbs deformation, (0.042± 0.11) short snout, (0.047± 0.047) microcephally, (0.095± 0.06) deformed tail and (1± 0.25) hematoma (Fig. 11).

International Journal of Scientific & Engineering Research Volume 6, Issue 3, March-2015
ISSN 2229-5518

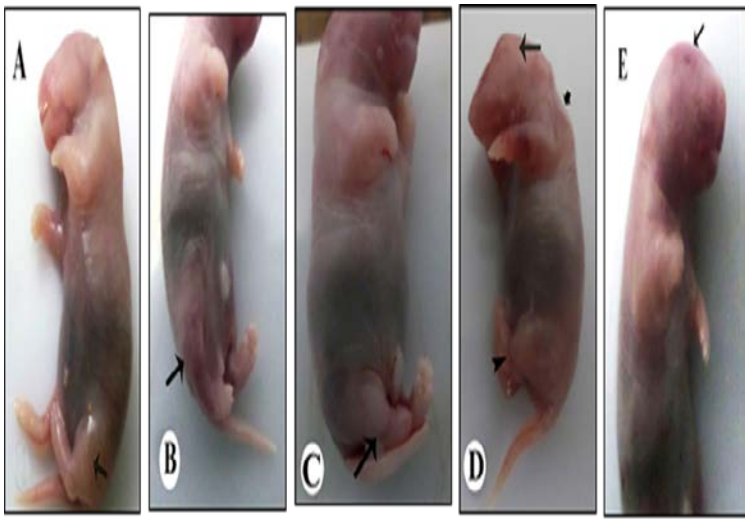


Fig. 9. Photographs of fetuses of maternally treated with 30.83 mg/Kg of mucophylline at 18th day of gestation. Showing: A) Fetus with club foot (arrow). B&C) Fetuses showed deformed hind limbs (arrow). D) Excephaly (arrow), abnormal curvature of bone back (short arrow) and (head arrow). E) Microcephaly (arrow).

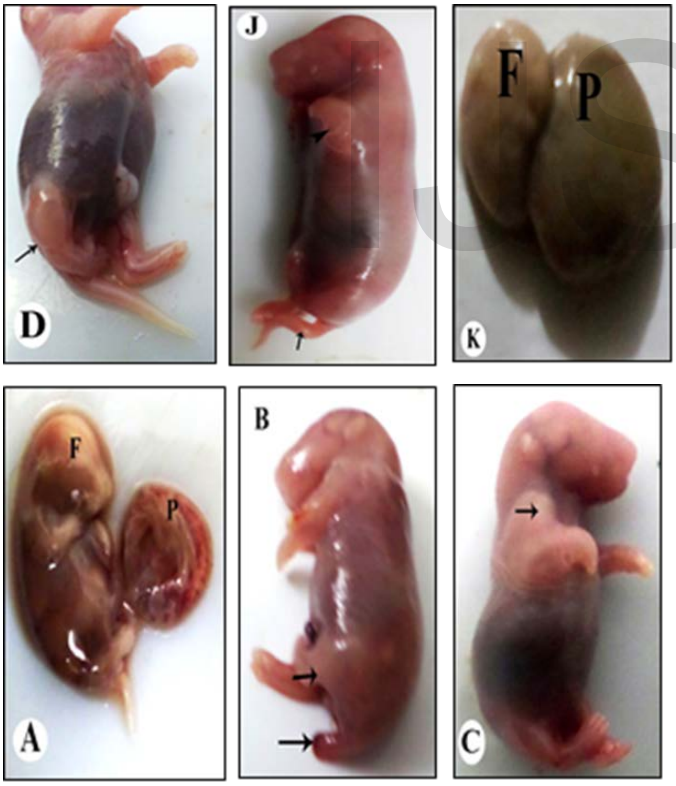


Fig. 10. Photographs of fetuses of maternally treated with 61.66 mg/Kg of mucophylline at 18th day of gestation. Showing: A) Transparent fetus. F= fetus, P= placenta. B) Fetus with club foot (short arrow) and cut tail (long arrow). C) Fetus show deformed forelimb (arrow). D) Deformed hind limb (arrow). J) Shortness of fore limb (head arrow) and kinky tail (arrow). K) Resorbed fetus. F= fetus, P= placenta.

Table 4. Showing effect of mucophylline on fetus morphology (gross pathological) at 18th day of gestation.

Groups	No. of examined fetuses	Hemato-ma	Hind limbs anoma-lies	Fore limbs anoma-lies	Short snout	Micro-ceph-aly	Tail anoma-lies
Control (A)	150	0.72 ± 0.22	0.27 ± 0.1	0	0.05 ± 0.05	0	0
Group (5^{th}-18) 30.83 mg/Kg (B)	98	0.1 ± 0.06a,b	0.65 ± 0.19b	0	0.25 ± 0.09	0.05 ± 0.05	0.1 ± 0.06
61.66 mg/Kg (C)	157	1.0 ± 0.25b	1.4 ± 0.22a,b	0.71 ±0.15a, b	0.042 ±0.11a	0.047 ± 0.047	0.095 ± 0.06

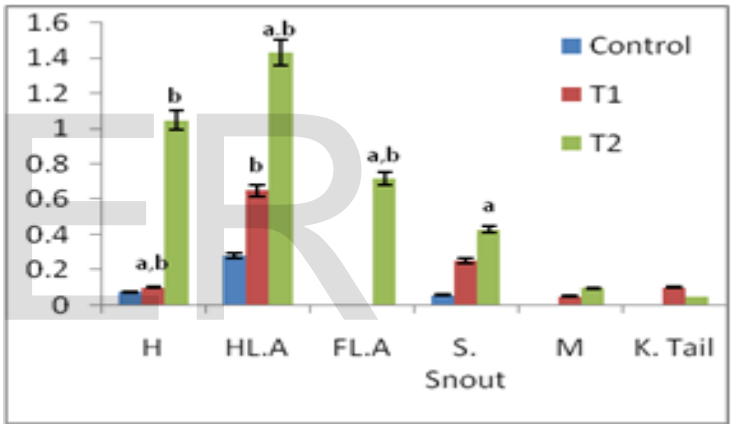


Fig. 11. Histogram showing gross morphology of fetuses at 18th day of gestation after oral administration of pregnant rats with different doses of mucophylline. Abbreviations: H= hematoma, HL.A= hindlimb anomalies, FL.A= forelimb anomalies, S.Snout= short snout.

3.7 Skeletal anomalies

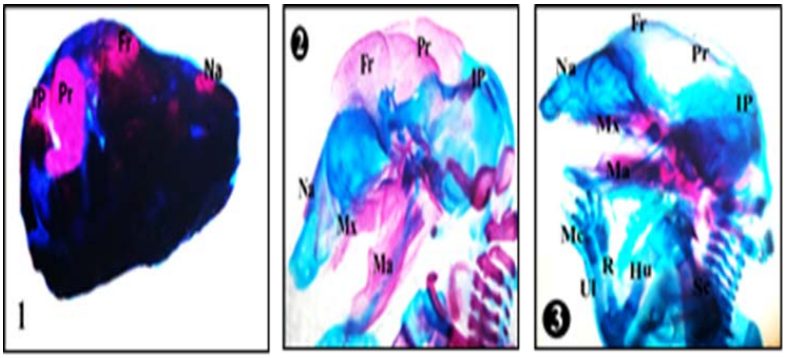
In general, the skeleton of the rat fetus at 18th day of gestation consist of two main parts; the axial and the appendicular skeleton. The axial skeleton contains the bones of skull, vertebral column, ribs and sternum. The appendicular skeleton comprises the bones of pectoral, pelvic girdles and fore, hind limbs. The skeleton malformations in all groups are represented in Fig. (12).

3.8 Control group

The skull elements of the control fetuses at 18th day of gestation are observed in a well ossified condition. The well ossified frontal and nasal bones cover the anterior portion of the brain. The zygomatic arch, squamosal bones and the posterior fontanella are well distinct. The posterior portion of the cranial roof is represented by interparietal bones (IP). The parietal bone

International Journal of Scientific & Engineering Research Volume 6, Issue 3, March-2015
ISSN 2229-5518

(Pr) represent by two large halves forming part of the roof and sides of the cranium. The premaxilla (Pm), maxilla (Mx), squamosal and zygomatic arch are the elements of the face and upper jaw. Both the cartilagenous supraoccipital and ex-occipital bones are separated by a gap called the occipital ring. This ring encloses the foramen magnum and forms the posterior wall of the cranial cavity. The vertebra is the basic structural unit of the vertebral column. The vertebral column is sub-divided into five regions, cervical, thoracic, lumbar, sacral ad caudal regions. The vertebral column of the normal fetuses consists from 7 cervical (Cr.V), 13 thoracic (Th.V), 6 lumbar (L.V), 4 sacral (S.V), and a variable number of caudal vertebrae depending on the tail length. In general, all the vertebral centra are fully ossified, but still surrounded by cartilaginous rings in caudal vertebrae. The neural arches are almost completely ossified except for a cartilaginous tip at fusion of the two arches.n, each vertebra consists of a ventral centrum and a pair of lateral neural arches. All fetuses have 13th pairs of well ossified ribs. Each rib consists of three rudiments: a cartilaginous tip lying proximal to the arch, an ossified middle portion and a distal cartilaginous portion. The sternum consists of six well ossified pieces called sternebrae. The pectrol girdle has two well ossified elements, dorsal scapula and suprascapula and ventral clavicles. The skeleton is built of well ossified bones; the humerus (Hu) supporting the upper arm (brachium); the radius (R) and ulna (Ul) support the lower fore arm (ant brachium) and the carpals (Ca), metacarpals (Mc) and phalanges (Ph) in the fore foot (manus). Each foot has 5 digits and has phalangeal formula 2:3:3:3:3. The metacarpals are partially ossified, while the carpals are still cartilaginous (Fig. 13). The pelvic girdle formed from two halves articulate ventrally at the pubic symphasis. Each half is made up of three elements; an anterodorsal bone called ilium (I), a posterodorsal one called ischium (Is) and a ventral flattened slender bone called os-pubis (Op). The skeleton of the hind limb is built up of the femur (Fe) supporting the thigh, tibia (Ti) and fibula (Fi) supporting the shank, and the tarsal (Ta) bones supporting the ankle. The metatarsals (MT) and phalanges are the bones of the hind paw. Each foot has 5 digits and has a digital formula 2:3:3:3:3(Fig. 14).



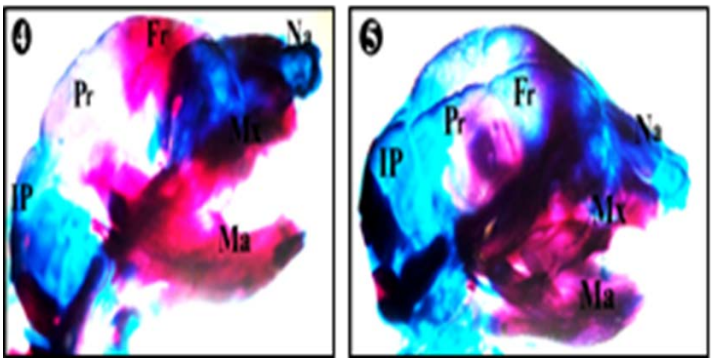


Fig. 12. Photographs of the cranial skeleton of fetuses at 18th of gestation. (Alcian blue & Alizarin red stain). 1) The cranial skeleton of control fetuses. Showing complete ossification of the cranial bones. 2&3) The cranial skeleton of fetuses maternally treated with 30.83 g/Kg. Showing incomplete ossification of all cranial bones (2) and unossified crainial bones (3). 4&5) The cranial skeleton of fetuses maternally treated with 61.66 mg/Kg. Showing incomplete ossification of all cranial bones. Na= nasal, Mx= maxilla, Ma= mandible, Fr= frontal, P= parietal and IP= interparieta.

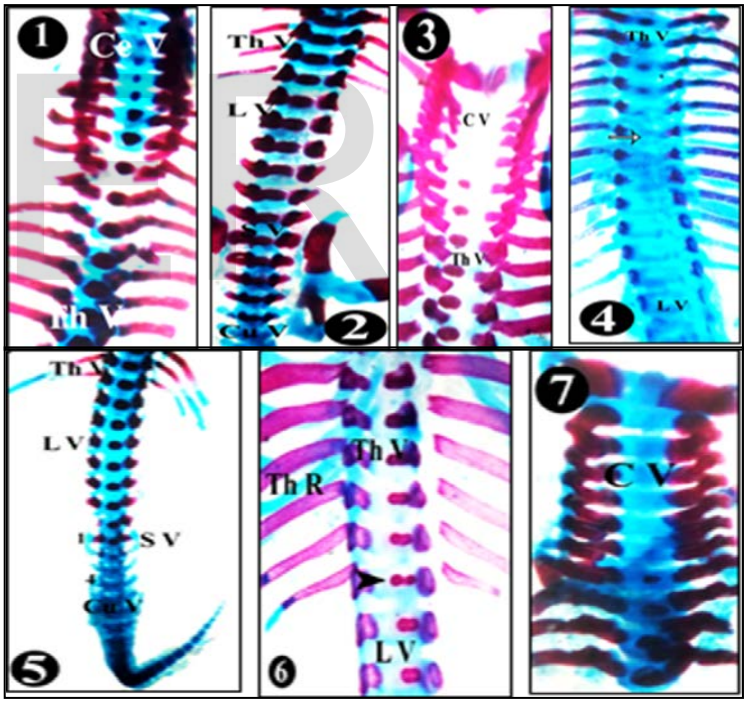


Fig. 13. Photographs of the vertebral column of fetuses at 18th of gestation (Alcian blue & Alizarin red stain). 1-3) The vertebral column of control fetuses. Showing complete ossification of all vertebrae. 4-6) The vertebral column of fetuses maternally treated with 30.83 mg/Kg. Showing unossified centers of cervical vertebrae (3), thoracic vertebrae (4) and sacral vertebrae and dumbelled shape vertebrae (6). 7) The vertebral column of fetuses maternally treated with 61.66 mg/Kg. Showing unossified centers of cervical vertebrae. Ce= cervical vertebrae, Th V= thoracic vertebrae, L V= lumbar vertebrae, S V= sacral vertebrae and Cu V= caudal vertebrae.

International Journal of Scientific & Engineering Research Volume 6, Issue 3, March-2015
ISSN 2229-5518

3.9 Treated groups

There were signs of skeletal anomalies in fetus skull from rats treated orally with 30.83 mg/Kg and 61.66 mg/Kg of mucophylline which represent in incomplete ossification of the bones in some fetuses and unossification of bones in other fetuses. Fetuses showed some vertebrae malformation such as, fine ossified vertebrae centra unossification of cervical vertebrae centra and dumbelled shaped vertebrae were seen. Ribs anomalies represent in, the wavy ribs, the rudimentary lumbar rib in the thoracolumbar region was observed, the costal separation (wide gap between ribs) was detectable, the slight ossification of ribs was also found (Fig. 15). Sternum anomalies such as unossification of all sternbrae or parietal ossification of some sternbrae (second and last one) were observed. Showed weakly degree of ossification was seen in scapula and bones of forelimbs, slight ossification of the 2nd, 3rd and 4th bones of the metacarpals and Lack ossification was seen in some bones (Fig. 16). Fetuses anomalies represented by slight ossification of the pelvic element, femur and tibia bones, fine ossification of the fibula, less ossified metatarsal bones and lack of ossification in metatarsal bones (Fig. 17&18).

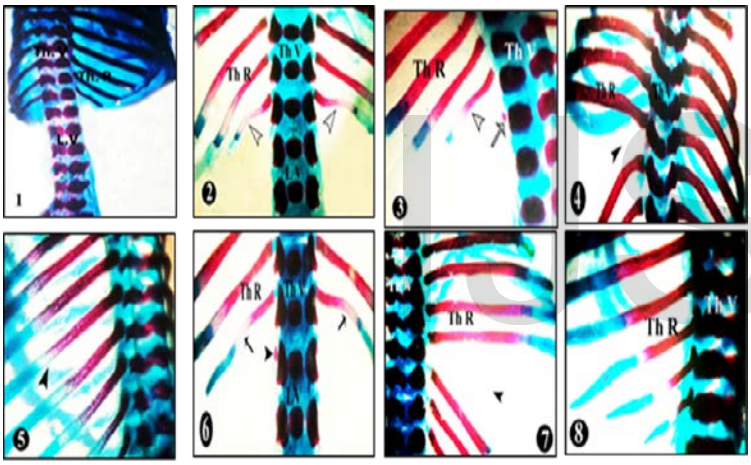


Fig. 14. Photographs of the ribs of fetuses at 18th of gestation. (Alcian blue & Alizarin red stain). 1) The ribs of control fetuses. Showing complete ossification and normal shape of ribs. 2-5) The ribs of fetuses maternally treated with 30.83 mg/Kg. Showing incomplete ossification, (2) curved ribs (head arrow), (3) wavy rib (head arrow) and rudimentary rib (arrow), (4) costal separation (head arrow), (5) incomplete ossification of ribs (head arrow). 6-8) The ribs of fetuses maternally treated with 61.66 mg/Kg. Showing incomplete ossification, (6) wavy rib (arrow) and rudimentary rib (head arrow), (7) costal separation (head arrow), (8) incomplete ossification of ribs. Th V= thoracic vertebrae and Th R= thoracic ribs.

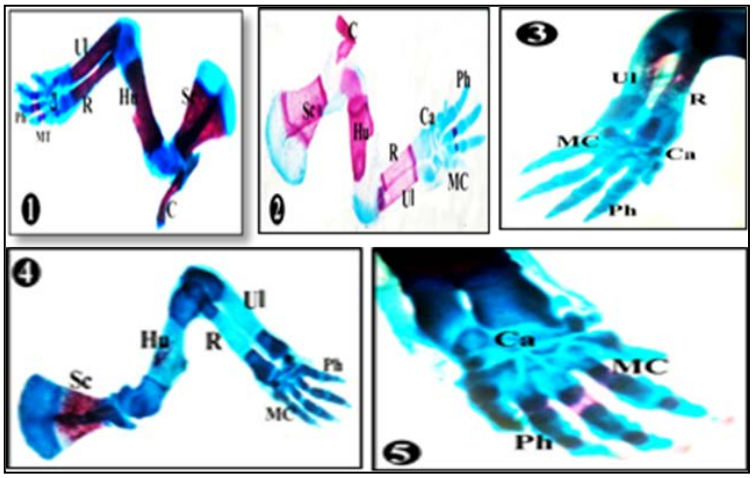


Fig . 15. Photographs of the sternum of fetuses at 18th of gestation. (Alcian blue & Alizarin red stain).(1) The sternum of control fetuses. Showing complete ossification of sternbrae bones. (2&3) The sternum of fetuses maternally treated with 30.83 mg/Kg. Showing unossified sternbrae bones (2) and the second and last sternbrae are unossified (arrow). (4&5) The sternum of fetuses maternally treated with 61.66 mg/Kg. Showing incomplete ossification of sternbrae bones (arrow).

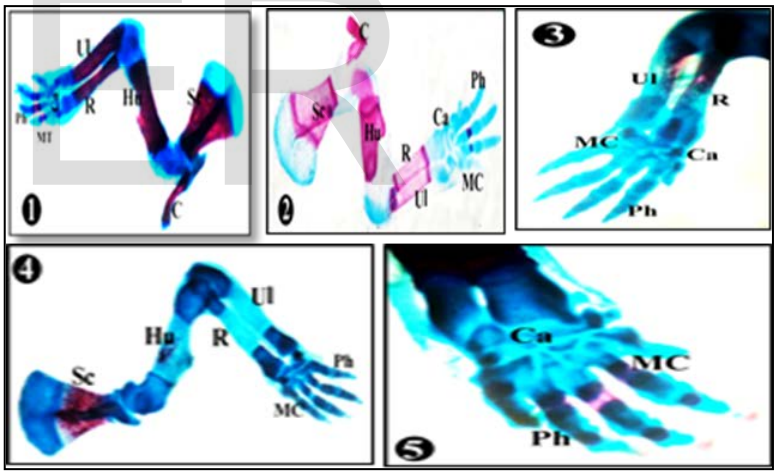


Fig. 16. Photographs of the pectoral girdle and fore limb of fetuses at 18th of gestation. (Alcian blue & Alizarin red stain). 1) The pectoral girdle and fore limb of control fetuses. Showing complete ossification of all bones. 2&3) The pectoral girdle and fore limb of fetuses maternally treated with 30.83 mg/Kg. Showing (2) incomplete ossification of all bones and (3) unossified MC and lack of ossification of R and U. 4&5) The pectoral girdle and fore limb of fetuses maternally treated with 61.66 mg/Kg. Showing (4) incomplete ossification of S and unossified H, R, U and MC and (5) incomplete ossification of MC. Sc= scapula, C =clavical, Hu=humerus, R= radius, Ul=ulna, Ca= carpals, MC= metacarpals and Ph= phalanges.

International Journal of Scientific & Engineering Research Volume 6, Issue 3, March-2015
ISSN 2229-5518

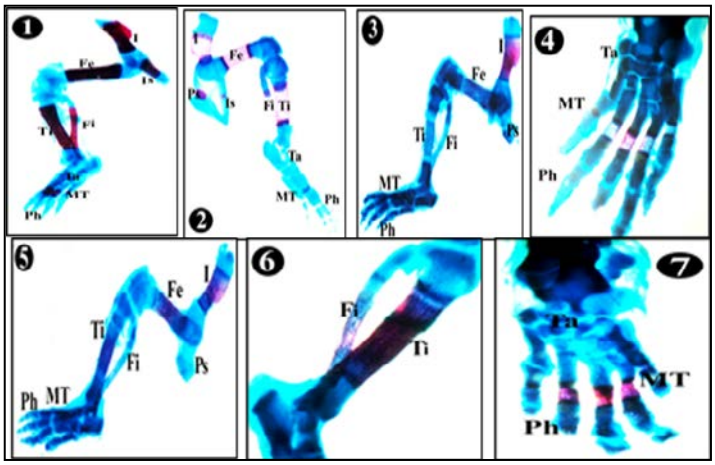


Fig. 17. Photographs of the pelvic girdle and hind limb of fetuses at 18th of gestation. (Alcian blue & Alizarin red stain). 1) The pelvic girdle and hind limb of control fetuses. Showing complete ossification of all bones. 2-4) The pelvic girdle and hind limb of fetuses maternally treated with 30.83 mg/Kg. Showing (2) incomplete ossification of all bones, (3) unossified Fe, T, F and MT and (4) incomplete ossification of MT. 5-7) The pelvic girdle and hind limb of fetuses maternally treated with 61.66 mg/Kg. Showing(5) incomplete ossification of I and unossified Fe, T, F and MT, (6) lack of ossification of T and F and (7) incomplete ossification of MT. I= ilium, IS= ischium, P=pubis, Fe= femur, Fi= fibila, Ti= tibia, Ta= tarsals. MT= metatarsals and Ph= phalanges.

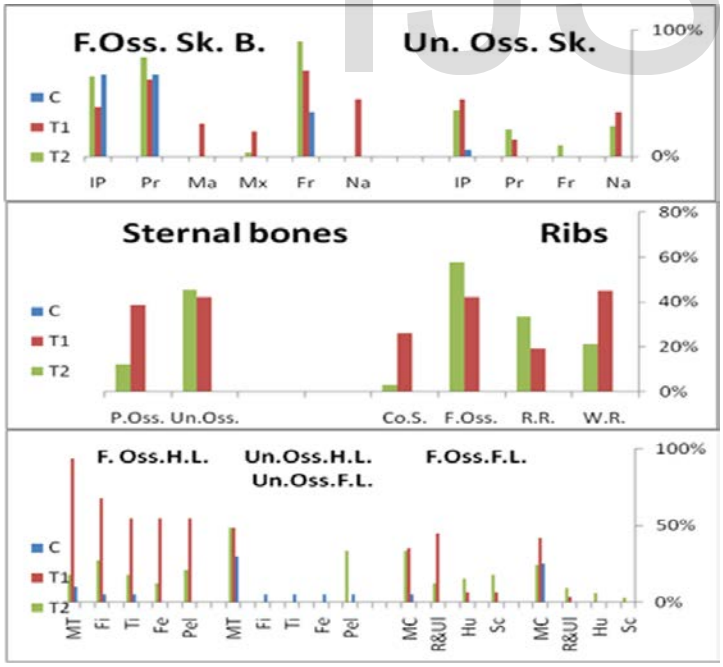


Fig. 18. Histogram showing the effects of mucophylline on the skeletal system of fetuses in treated rats at 18th day of gestation. F.Oss. = fine ossified, Un.Oss. = unossified, H.L. = hind limb, F.L. = forelimb & Sk.B. =skull bones.

4 Discussion

Toxic effects on embryo are experimentally obtained by administering the agent on the mother. The drug is one of such type of agent, which produces toxicity to the mother, and an effect would be expected in the intrauterine environment surrounding the fetus. It is a general principle that the administration of any drug to a pregnant patient is to be avoided, because of possible fetal damage. Development of the embryo is affected by teratogens mostly during the process of organogenesis, which is recognized as the time period from the occurrence of the neural plaque to closure of the plate. It begins usually on the 18th –21st days in human being, prolongs nearly 36 days. Likewise, it starts on the 6th day in rats, going on for 10 days (Vickers and Brackley, 2002). During this process, teratogenic agents can lead significant congenital anomalies.

Administration of mucophylline via oral intubation during the period of organogenesis exhibited decrease in weight of the pregnant mice. Fetal development retardation and embryo fetotoxicity were observed as evident by the reduction in fetal weights, number of live fetuses, and higher incidences of resorptions and postimplantation death.

Exposure also increased the fetal as well as skeletal anomalies. A reduction in maternal weight was observed in the treated group. No mortality occurred in any experimental groups. This decrease in maternal weight can be correlated to decrease in diet consumption and water intake. (Lindström et al., 1990) also observed similar phenomena in rats treated with theophylline. However, this alone cannot contribute to decreased maternal weight. It could also possibly be due to increased degeneration of lipids and proteins leading to decreased organ weight as a result of drug toxicity. Other factors may also be responsible for the reduced maternal body weight, which could probably be due to mucophylline-induced resorptions, decreased weight, and growth of the fetus. The average number of live fetuses per dam was another parameter, which was adversely affected after mucophylline administration. A concurrent rise in the number of resorbed and dead embryos/fetuses along with reduced number of live fetuses per dam was observed. Thus, the evident decline in the number of live fetuses per dam is probably due to increased percentage of resorbed embryos. Similar results were also obtained by several authors (Lamb et al., 1997 and Morrissey et al., 1988), who observed a decrease in number of live fetuses due to an increased number of resorptions in mice treated with theophylline.

Embryotoxicity and fetotoxicity of theophylline has been observed in mice and rats, by several authors (George et al., 1986). A dose-dependent decline in fetal weight was observed in all the experimental groups. This correlates well with the decrease in maternal weight.

Decrease in fetal body weight is a sensitive and precise indicator for growth retardation.The plausible cause of such an association may be due to maternal organism being under stress, which in turn might affect the growing fetuses leading to its growth retardation and hence reduced weight of the fetuses. New and Coppola (1977) reported that acute interruption of blood flow to the uterine horn caused growth retardation of the fetuses.

International Journal of Scientific & Engineering Research Volume 6, Issue 3, March-2015
ISSN 2229-5518

Reduction in the uterine vascularization subsequently decreases blood flow to the uterine horn and this induces fetal-placental growth retardation (Garris, 1984). The reduced weight of the fetuses was accompanied by incomplete ossification of the fetal skeleton, which may be one of the factors for growth retardation leading to reduced weight of the fetuses. Further, deformities in limb such as club foot were evident after mucophylline administration. Club foot formation can possibly be due to (i) indirect action of mucophylline, (ii) alteration of maternal physiology, which disturbs the hormonal balance in mother, or (iii) direct effect on the tissue primordial of foot (Sharma et al., 2001). Similar were the observations with theophylline (Tucci and Skalko, 1978).

Because of the fetal rodent skeleton at term is only partially ossified single staining of bone cannot accurately describe normal and abnormal fetal skeletal structures. Inspite of these limitations the single stain for bone is universally used in routine teratology tests, as it is simpler and cheaper than double staining methods (Menegola et al., 2002). Although these unossified structures generally become ossified as development continues, Alizarin Red S does not specifically stain these cartilaginous precursors of bone, and this technique is not useful for specific identification of cartilage (Burdan et al., 2002 and Menegola et al., 2002). Failure to evaluate the cartilaginous portions of the skeleton may result in failure to identify important abnormalities in skeletal morphology. The double staining method for fetal skeletons was proposed several years ago by different investigators, with minor differences in methodology (Soysal et al., 2011).

Double stained skeletons of exposed fetuses revealed reduced ossification of skull bones (nasal, frontal, parietal, intraparietal, and supraoccipital), reduced/wavy ribs, reduced/bifurcate sternebrae, reduced number of pelvic elements, reduced ossification/absence of carpals, metacarpals, tarsals, metatarsals, phalanges, and caudal vertebrae, and displaced/absence of vertebral centra in thoracic, lumbar, and sacral regions. This reduced ossification of various bones may be due to altered calcium metabolism or decreased calcium and magnesium ion levels as well as altered calcitonin level in the growing fetus, thereby causing retardation in bone development.

5 References

Burdan, F.; Rozylo-Kalinowska, I.; Rozylo, T.K. and Chahoud, I. (2002). A new, rapid radiological procedure for routine teratological use in bone ossification assessment: a supplement for staining methods. Teratology, 66: 315–325.

De Swiet, M. (1977). Diseases of the respiratory system. Clin. Obstet. Gynaecol. 4:287-96.

Fujii, T.; and Nishimura, H. (1969). Teratogenic actions of some methylated xanthines in mice. Okajimas Folia Anat. Jpn., 46: 167-175.

Garris, D. R. (1984). Intrauterine growth of the guinea pig fetalplacental unit throughout pregnancy: regulation by uteroplacental blood flow. Teratology, vol. 29, no. 1, pp. 93–99.

George, J.D.; Price, C.J.; Marr, M.C.; and Kimmel, C.A. (1986). Developmental toxicity of theophylline (THEO) in mice and rats. Teratology, 33:70C-71C.

Gordon, M.; Niswander, K.R.; Berendes, H. and Kantor, A.G. (1970). Fetal morbidity following potentially anoxigenic obstetric conditions: VII. Bronchial asthma. Am. J. Obstet. Gynecol., 106:421-9.

Lamb, J., Gulati, D., Chambers, R., Shaver, S. and Sabharwal, P. (1997). Reproductive toxicology. Theophylline. Environ. Health Perspect., 105(1): 1355-1356.

Lindström, P.; Morrissey, R.E.; George, J.D.; Price, C.J.; Marr, M.C. and Kimmel, C.A. (1990). The developmental toxicity of orally administered theophylline in rats and mice. Fundam. Appl. Toxicol., 14: 167-178.

McClain, R.M. and Becker, B.A. (1975). Teratogenicity, foetal toxicity and placental transfer of lead nitrate in rats. Toxicol. Appl. Pharmacol., 931: 72-82.

Menegola, E., Broccia, M. L.;Di Renzo, F. and Giavini, E. (2002). Comparative study of sodium valproate-induced skeletal malformations using single or double staining methods. Reprod Toxicol., 16(6): 815-23.

Morrissey, R.E.;Collins, J.J.; Lamb, J.C.; Manus, A.G. and Gulati, D.K. (1988). Reproductive effects of theophylline in mice and rats. Fundam. Appl. Toxicol., 10: 525-536.

New, D. A.; and Coppola, P. T.; (1977). Development of a placental blood circulation in rat embryos in vitro. Journal of Embryology and Experimental Morphology, vol. 37, pp. 227–235.

Peters, (1977). Double staining of foetal skeletons for cartilage and Bone in:" Methods in prenatal toxicology ". Neu. bert,D.; Merkerd, H.J. and Kwasigroch,T.E. George. Thieme. Stuttgart., 153-154.

Schaefer, G. and Silverman, F. (1961). Pregnancy complicated by asthma. Am. J. Obstet. Gynecol., 82:182-91.

Sharma, S., Mathur, U.; Saini, M. R.; Sharma, M.K., Kumar, M. and Kumar, A. (2001). Fetal malformation in Swiss albino mice on exposure to lead during gestation period. Bulletin of Environmental Science, vol. 19, no. 200, pp. 81–84.

Soysal, H.; Unur, E.; Duzler, A.; Karaca, O. and Ekinci, N. (2011). Effects of intraperitoneal administration of the phenytoin on the skeletal system of rat fetus. Seizure, 20: 187–193.

Vickers, M. and Brackley, K. (2002). Drugs in pregnancy. Curr. Obstet. Gynaecol., 12: 131–7.

- **Author**: Abdel Wahab El-Ghareeb, Department of Zoology, Faculty of Science, Cairo University- Egypt.
- **Co-Authors:** Hamida Hamdi, Zoology department, Faculty of Science, Taif University, Saudi Arabia, EL-Sayed Fahim Taha and Heba Ali, Department of Zoology, Faculty of Science, Cairo University- Egypt.