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SODIUM DICHROMATE EFFECT ON REPRODUCTIVE SYSTEM OF RATS AND THEIR FETUSES AND PUPS

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ABSTRACT
This study was performed in order to evaluate the changes induced by sodium dichromate (SDC) in rats treated orally with two doses T1at (3mg/kg B.W.) and T2 at (9mg/kg B.W.) However, the control group dosed daily with distilled water. Developmental study was performed in which (210) rats were used 140 female and 80 male divided equally into three pregnancy phase treatment P1, P2 and P3 groups according to the exposure developmental growth period during pregnancy that subdivided equally to T1P1, T1P2, T1P3, T2P1, T2P2, T2P3 according to the SDC dose used. One group dosed with distilled water during all pregnancy phases to be considered as control group. The Subgroups T1P1, T2P1 were treated male for one month and fourteen days for female before mating, then after pregnancy continues female dosing during the first pregnancy phase (from1-5days). While T1P2,T2P2 subgroup were treated during the second trimester (6-15days). Whereas, the third treatment groups were T1P3, T2P3 were treated from (15-21days) of the pregnancy. Half number of each treated subgroups were sacrificed one day before parturition to study the prenatal effect on their fetuses. While the other half of treated pregnant rats were left after delivery during lactation (3weeks) to study postnatal effect on their suckling pups. The prenatal effect showed that the fertility index recorded a remarkable decrease percent in group T2P1 (12.5%), T1P1 (16.6%) compared with control one (83.3%). While the gestation index of the females (of the treated groups) were high at T1P2 (90%), low at T2P3 (65%) respectively in comparison with the females of control one which recorded (100%). Also T1and T2 groups have recorded dose dependent increase in the percentage of resorbed fetuses and total anomalies due to SDC oral dosed given before and during pregnancy in compares with control group. The postnatal effect showed variable body weight change effects in growth of pups along the period of the study showing a significant decrease ($P<0.05$) with highest effect inT2P3 subgroup in comparison with control group. The results of Viability index showed remarkable dose dependent decrease especially in T1P3, T2P3group in comparison with the control one respectively especially in the first day after delivery .The effect of SDC on Lactation index recorded a dose dependent decrease results in T1P3 T2P1, T2P2 and T2P3 in comparison with that of T1P1, T1P2 and control groups .The highest significant decline in (L.I.) was recorded in T2P3 (88.7%) as compared with other and control groups respectively.

KEYWORDS: sodium dichromate (SDC), fertility index, variable body weight.

INTRODUCTION

Chromium is found in rocks, soil, gases, animals and plants. The steadiest forms are chromium (0), chromium (III) and chromium (VI), (IPCS, 2013). Chromium(VI) compounds produced by the chemical produce which used in a lot of applications, including chrome plating, wood preservatives, the manufacture of dyes and pigments, surface coatings and corrosion inhibitors (ATSDR, 2012). Chromium (VI) is known to cause problems. When it is a compound in leather products, it can cause allergic reactions, such as skin rash. After breathing it can cause nose irritations and nose bleeds, upset of stomachs and ulcers and respiratory problems. It weakened the immune systems, kidney and liver damage, alteration of genetic material, lung cancer and death. The absorbed hexavalent chromium is distributed throughout the body, blood, bone, testis, brain, breast milk and uterus (McGraw-Hill, 2003 and Costa and Klein, 2006). The chromium manufacturing caused pollution to the environment which leads to toxic actions, although much of the damage may be due to its intracellular reduction to the even more highly reactive and short-lived chemical species Cr (III) and Cr (V). Exposure to Cr (VI) can result in different point mutations in DNA and to chromosomal damage, plus to oxidative changes in proteins. The importance of chromium effects by free oxidizing radicals they may produce in the body and causing cancers and allergic sensitization remain to be demonstrated.(James and Stephen, 2004) . Because of contradictive and lack of some information about this highly toxic material SDC and its effects on reproductive system for both sexes and their pups (rats) Developmental study designed according to FDA which aimed for the following:

1. Study the effects of SDC on reproductive and fertility of male and females at different doses in rats.
2. Study the effect of SDC on development (prenatal and postnatal) in pregnant female rats according to dose and phase of exposure.

MATERIALS & METHODS

Experimental animals

At this study (210) male and female albino rats with ages about three months and body weight ranged between (150-200g) were used with their pups to perform the three experiments of the present study .The animals were raised and bred in the animal house of College of veterinary medicine/ University of Baghdad where the research was done. The animals were kept in cages of (20x30x50) cm^3 dimensions in average of three rats in each cage one month before study for acclimatization in optimum conditions of breeding at (22±3) ºC with a (14/10) Hours (Light/Dark) cycle .Commercial feed pellets and drinking water were given all the time of experiment (Hafes, 1970).

Insurance of Pregnancy

The pregnant female rats examined daily after mating for five days. Vaginal smear was prepared by using vaginal swabs with cotton on the head and Methyline blue stain to detect proestrus phase. Pregnancy detected by observation pale mucous membrane of vagina and sperms in the third day after conception (Hafes, 1970).

The dose of experiment depend on oral LD_{50} of male rat that reported by (Gad *et al.*,1986) represented 0.1 of LD_{50} of SDC, and it is three fold dose by dissolved 30mg of SDC in 10ml of D.W. the concentration was 3mg/ml while the dose was 0.1ml/100g B.W.

Experimental design of developmental study

140 female and 70 male rats were divided into different treated groups according to the exposure developmental period during pregnancy and the dose of SDC used in accordance with registered design of FDA (klassen *et al*., 2008).

Phase 1 treated groups (P1): Represent the exposure before mating for male and female and at the early phase of pregnancy in which highly cellular division and blastocyst implantation in order to study the cytogenic reproductive and fertility effect.

A group of 48 female rats and 12 male that divided equally in to those treated with SDC. At dose T1(3mg/kg B.W) and three fold dose of SDC at (9mg/kg B.W) represented T1P1and T2P1respectively that given orally daily for (14) days for female and one month for male and after successful mating dosing continue in pregnant female for the period 1-5 days.

Phase 2 treated group (P2): Represented the exposure of pregnant female to SDC at the 2nd period of pregnancy in which organogenesis and highly developmental growth may occur due to exposure to SDC and major teratogenic effect.

A group of 48 pregnant female rat divided equally (24 rats) for each treated groups T1,T2 at (3mg /kg B.W) and three fold SDC dose (9mg/kg.bw) represented T1P2and T2P2respectively given orally daily for the period 6-15 days of pregnancy .

Phase 3 treated groups (P3): represented the exposure of pregnant female to the SDC at the period of functional growth and minor developmental effect in order to study the functional effect and fetal growth at the third period of pregnancy exposure.

A group of 48 pregnant female rat were divided equally 24 rats each in the those treated with (3 mg/kg.bw) and three fold SDC dose (9 mg/kg.bw) given orally daily for the period 16-21 days of pregnancy represented T1P3, T2P3 respectively. Half of pregnant female from each phase treated subgroups were sacrificed one day before delivery in order to study the prenatal effect, the other half female were left for delivery and stopped dosing after parturition in order to study the postnatal effect.

Control group(C): Control group consists of 24female rats and 12 male one were subjected to daily orally dosing with D.W .for the period of 30 days for the male rats and 14 days for females before mating and throughout pregnancy and lactation period.

1 Parameters of study

1-Fertility and reproductive indexes for T1P1and T2P1 and control groups according to following equation (Klaassen *et al*., 2008).

$$\text{Fertility index} = \frac{\text{No. of successful mating resulted in pregnancy}}{\text{Total No. of mating}} x100$$

$$\text{Gestational index} = \frac{\text{No. of pregnancy that give full term with life birth}}{\text{Total No. of pregnancy}} x100$$

2- Prenatal effect

a. No. of dead ,alive ,and resorbed fetuses in the uterus.

b. Anomalies in soft and hard tissues of live fetuses.

3- Postnatal and lactogenic (nursing) effects

4- Weight and numbers of a live neonate at first day after delivery.

5-Viability index $= \frac{\text{No.of neonate that survive 4 days lactation}}{\text{Total No.of neonate}} x100$

6-Lactation index $= \frac{\text{No.of neonate that survive 4 – 21 days lactation}}{\text{Total No.of neonate at 4 days of lactation}} x100$

7 - Pups weight change (weekly) during lactation

8 - Observing anomalies. Clinical signs in developing pups.

Statistical Analysis

The statistical analysis done by using the (SPSS 14.0, 2012) data was given in the form of arithmetical mean values and standard errors. Two-way and one-way analysis of variance was performed .Groups differences were determined by using LSD test.

RESULTS

1. Fertility index for T1P1, T2P1and C

The fertility index as listed in table (1) showed significant decrease ($P<0.05$) at group T2P1 (12.5%) lesser than that of T1P1 (16.6%) and control one (83.3%).

TABLE 1: showed the Fertility index in T1P1and T2P2 in SDC developmental effect study in rats

Group	NO. of successful mating resulted in pregnancy	Total number of mating	Fertility index %
T1P1	20	120	16.6%
T2P1	18	144	12.5%
control	20	24	83.3%

T1P1= Daily SDC dose at 3mg/ kg.BW during and before phase one of pregnancy (1-5) days
T2P1 = Daily SDC dose at 9 mg / kg.BW during and before phase one of pregnancy (1-5) days
C= control group dosed daily with distal water

2. The effect of sodium dichromate on Gestational index

The results of gestation index which is the number of pregnant females that give normal birth had revealed profound significant decrease ($p<0.05$) in gestation index of females of both treatment groups in comparison with the females of control one that recorded (100%). The results revealed marked decline in gestation index of both females of T1P1 (75%) for T1groups and T2P2 (70%), T2P3 (65%) for T2 groups in a manner positively proportional with their SDC doses before and during pregnancy periods, table(2).

TABLE 2: showed the gestational index in SDC treated and control groups dosed before and during pregnancy according to gestation period exposure subgroups

Group	No. of pregnant females	No. of pregnant females resulted life off springs	No. of pregnant females resulted dead off springs	Gestational index %
T1P1	12	9	3	75%
T1P2	12	11	1	91.6%
T1P3	12	10	2	83.3%
T2P1	12	9	3	75%
T2P2	12	8	4	66.6%
T2P3	12	8	4	66.6%
C	12	12	0	**100**%

T1P1= Daily SDC dose at 3mg/ kg.BW dose before and during phase one (1-5) day of pregnancy
T1P2= Daily SDC dose at 3mg/kg BW .during phase two (6-15) day of pregnancy.
T1P3= Daily SDC dose at 3mg/kg BW. during phase three (16-21) day of pregnancy
T2P1 = Daily SDC dose at 9 mg / kg.BW before and during phase one (1-5) day of pregnancy.
T2P2= Daily SDC dose at 9mg/kg BW .during phase two6-15) day of pregnancy
T2P3= Daily SDC dose at 9mg/kg BW. during phase three (16-21)day of pregnancy
C = control group dosed with distilled water

Prenatal effects

1 -Number of dead, alive and resorbed fetuses

Table (3) showed percentage of resorbed and dead fetuses on SDC treated subgroups which recorded in T1, T2 sacrificed female before birth, T1 subgroup dosed orally with 3mg/kg B.W during different gestation periods and sacrificed before parturition, showed that the total number of dead and resorbed fetuses were (72) fetuses at T1P1subgroup,the females showed grossly (14) dark spots of resorbed fetuses adhered to the uterus (19.4%).

TABLE 3: showed percentage of resorbed and dead fetuses by SDC

Groups	No. of sacrificed female rats	Total No. of fetuses	No. of resorbed fetuses	% of resorbed fetuses	No .of dead fetuses	% of dead fetuses
C	12	120	0	0%	2	1.66%
T1P1	12	72	14	19.4%	7	9.72%
T1P2	12	77	8	10.38%	5	6.49%
T1P3	12	60	5	8.33%	7	11.66%
T2P1	12	76	16	21.05%	9	11.84%
T2P2	12	55	12	21.81%	13	23.63%
T2P3	12	59	15	25.42%	17	28.81%

Fig. (1) .While there were (7) dead fetuses recorded (9.72%).T1P2 subgroup recorded total number of fetuses (77) with (10.38%) resorbed fetuses and (6.49%) dead fetuses .The T1P3 recorded (8.33%) resorbed fetuses with 11.66% dead fetuses. While in T2 subgroup in which their females were orally dosed with 9mg/kg B.W. of SDC during different gestation periods ,the number of females involved were (12) females for each subgroup which produced atT2P1 subgroup 76fetuses ,showed(21.05%) resorbed fetuses and(11.84%) dead fetuses.T2P2 subgroup with 55 fetuses recorded (21.81%) resorbed fetuses, (23.63%) dead fetuses. While T2P3 subgroup (59) fetuses with (25.42%) resorbed fetuses and (28.81%) dead fetuses. These results showed a significant difference ($p<0.05$) in comparison with the control group which recorded (0%) resorbed fetuses and (1.66%) dead fetuses.

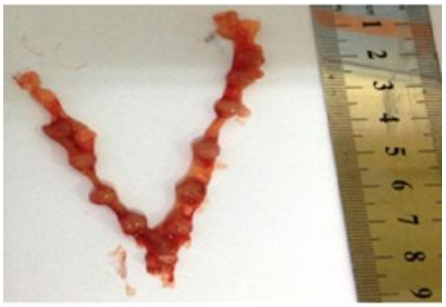

FIGURE 1: showed uterus with resorbed fetuses at T1P1 group

The results of Ratio of organ to body weight of rats groups during different gestation periods orally dosed SDC (prenatal**)** were recorded highly marked decrease at spleen, kidney, liver and brain at T2P2 subgroup compared to control and other subgroups, while the uterus was at subgroup T2P3 less weight ratio than other sub group and control one .That is due to changes in reproductive organs and cytogenetic effects results confirm such effect proportional with administration SDC dose.

TABLE 4: Showed the ratio of organ to body weight of rats groups during different gestation periods orally dosed SDC / prenatal

Organs groups	control	T1P1	T1P2	T1P3	T2P1	T2P2	T2P3
Spleen	0.00740±0.000	0.0044±0.000	0.0045±0.0001	0.0045±0.0001	0.004±0.00017	0.0034±0.000	0.00414±0.000
LSD=0.001	13	15	3	4	B	15	92 CD
74	A	B	B	B		E	
Kidney	0.0108±0.0001	0.0083±0.000	0.00826±0.000	0.00784±0.000	0.00848±0.000	0.0072±0.000	0.0072±0.0001
LSD=0.002	1	16	15	12	14	15	5
24	A	B	B	C	B	C	C
Liver	0.0648±0.0003	0.0508±0.000	0.0426±0.0014	0.0617±0.0021	0.0441±0.001	0.0396±0.001	0.048±0.00043
LSD=0.015	9	53	B	A	B	2	B
4	A	B				CB	
Brain	0.00828±0.000	0.0074±0.000	0.0066±0.0001	0.0072±0.0000	0.00612±0.000	0.0061±0.000	0.0067±0.0005
LSD=0.001	15	1	2	5	14	17	B
49	A	A	B	A	B	B	
Uterus	0.0144±0.0003	0.0107±0.000	0.0116±0.0001	0.0105±0.0001	0.0104±0.0003	0.0096±0.000	0.0094±0.0001
LSD=0.003	9	4	6	4	7	44	B
76	A	A	B	B	B	B	

Anomalies and deformities

The results showed dosed that fetuses in T1P3 and T2P3 subgroups suffered from subcutaneous hemorrhage patches diffused in abdominal, hind limbs and around nostrils with deformation of legs or hands Fig. (2 , 3) & table (5).

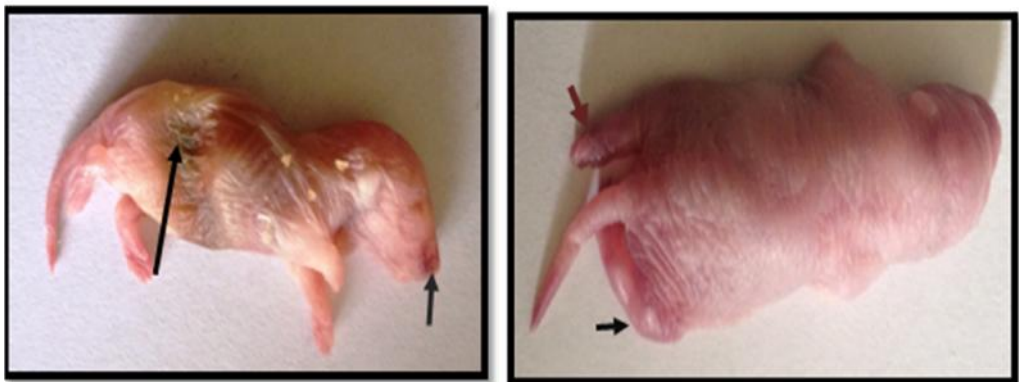

FIGURE 2: showed pups with subcutaneous hemorrhage T1P3 group
FIGURE 3: showed pups with subcutaneous hemorrhage T2P3 group & leg deformate

TABLE 5: Anomalies effects recorded in SDC dosed subgroups during different stage of pregnancy

groups	Total No. of fetuses and	Subcutaneous hemorrhage	Subcutaneous hemorrhage%	Deformation of the leg or	Deformation of the leg or	Total % of Teratognesis

	neonates			hands	hands%	
T1P1	72	5	6.9	2	2.7	9.6
T1P2	77	10	12.9	5	6.4	19.3
T1P3	60	8	13.3	4	6.6	19.9
T2P1	76	13	17.1	8	10.5	27.6
T2P2	55	6	10.9	3	5.4	16.3
T2P3	59	11	18.6	6	10.1	28.7
Control	120	0	0	0	0	0

DISCUSSION

The results of prenatal developmental study in T1 and T2 groups revealed that there were significant increase in the percent of resorbed fetuses and percent of total anomalies positively proportional with sodium dichromate oral dosed given before and during pregnancy. Those results indicate that SDC passed the placenta and affect all developmental stages beginning with implanted ovum since there were high percent of resorbed fetuses recorded in T2and to lesser effect in T1. Failure of fertilized ovum implantation at stage (1) pregnancy resulted in early embryonic death that manifested as resorbed fetus. Also the high percent anomalies recorded mainly in T2 and lesser extent in T1 indicated that the effect were in stage (2) and stage (3) pregnancy since the effect were structural anomalies as recorded in stage 2 that consider the main teratogenic period while retard growth and possible functional effect may represent stage 3 pregnancy effect as reported by Kirpnick-sobol *et al. (*2006)

The reported effect of SDC on red blood cells which cause anemia that lead to inadequate blood supply and nutrition reached to embryo as well direct toxic effect of transferred SDC through placenta and possible caused death of embryo or fetuses (NTP, 1996and Bucher, 2007). Kanojia *et al*. (1996) observed significant increases in pre-implantation and post implantation losses and additional effects included increases in abnormalities in tail and wrist forelimbs, and sub dermal hemorrhagic patches in the off spring. Also the rapid crossing of sodium dichromate to placental barriers may cause direct toxic effect to embryo and fetuses manifested by prenatal death (resorption) and anomalies according to Samuel *et al*. (2012). These findings agreed with the preceding studies of Elsaieed and Nada (2002) who found an increase in the incidence of re-sorption at pregnant rats that received oral dose of Cr(VI),also this result agreed with those of Junaid *et al*. (1996) who exposed females Swiss albino mice and rats to dichromate salts orally and reported retarded fetal development, embryo and fetotoxic effects including reduced fetal weight, reduced number of fetuses (live and dead)per dam ,and higher incidences of stillbirths and post–implantation loss. Chromium compounds are widely distributed in body compared to chromium (III),reflecting the greater tendency of chromium (VI)to cross plasma membranes (IPCS, 2006).Chromium (VI) is unstable in the body and is reduced to chromium (V), chromium(IV) and ultimately to chromium (III) by endogenous substances such as ascorbate and glutathione and believed that the toxicity of chromium may result from damage to cellular components during this process *e.g.* through the generation of free radicals causing depletion of these antioxidants in the tissue by chromium reduction (Defra and EA, 2002).

The prenatal teratogenic effect included subcutaneous hemorrhages in various parts of fetuses' body and dead fetuses were recorded in all treated subgroups (T1, T2) accordingly with the dose. This phenomenon can be explained by the accumulation of chromium in the body due to its prolonged half-life: chromium remains bound to hemoglobin and glutathione in the red blood cells while they are pumped to different parts of the body. Naturally, fetuses have higher hemoglobin levels than their mothers and are more liable to chromium accumulation (Saxena *et al.*,1990).The chromium-hemoglobin complex is relatively stable and remains within the cell over life span of erythrocyte, leading to microcytic, hypochromic anemia NTP (2008). While the gestation index is an important parameter for assessment or studying the teratogenic effects and intrauterine development of fetus (Al-Hamood, 2003).

The results of gestation index showed that SDC in oral dose 1 and 3 fold mg/kg B.W. have significant effects on maternal reproductive system and embryo life which marked in pregnant females exposed with their male to 1and 3 fold of SDC dose that registered the lowest gestation index (75-65%) respectively which indicated that dose depended effect may be due to direct toxicity of SDC on ovulation, fertilization, embryo implantation as well as due to cytogenetic effects in male and female of T1P1, T2P1subgroupsThe result of fertility index of both T1 and T2 groups revealed such dose dependent and cytogenetic effect the reproductive performance and effect successful fertilization and implantation in our study high dosed pregnant rats of T2 groups which were received 3 fold of SDC (9mg/kg B.W.). All these results affect the reproductive success of exposed workers and laboratory rats. Sakhila (2008) reported during pregnancy this prolongated gestation period which attributed to inhibition of prostaglandins synthesis which play important role in cervical dilation and uterine contractility and fetal adaptation to labor. Chromium (VI) disrupts the ordered functions of the placenta, which leads to reproductive disorders in rats .Our results indicated that SDC seemed to affect fertility and reproductive performance in dose dependent manners confirmed by results of fertility and gestation index and can passed to placenta and affect all developmental stage, beginning with oval implantation to continue in second and third phase of pregnancy causing structural and functional defect of their fetuses and neonate while the SDC postnatal effect was the most obvious in the first two days of neonate lives and to lesser extent in second week of lactation period in dose dependent manner indicating the structural and functional

change of fetuses during second and third pregnancy phase that effecting the survival of their pups at these critical lactation periods

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