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Biological Mechanisms of Lithium separately and in combination with Caffeine in Palatogenesis in Albino Rat

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ABSTRACT

Purpose: The purpose of this study was to evaluate the Biological Mechanisms of Lithium separately and in combination with Caffeine in Palatogenesis in Albino Rat **Materials and Methods:** Thirty six adult rats were used in this study, twenty four female and twelve male rats, after mating procedures and confirmation of pregnancy the pregnant rats were divided into 3 groups :group1(control) **,** which consists of six pregnant rats and were not receive any medication during gestation, group2' Which consists of nine pregnant rats and were received prianil (lithium carbonate) 200mg/kg intragasteric During the period of (6th to 15th) day of gestation &group 3: Which consists of nine pregnant rats and were received prianil (lithium carbonate) 200mg/kg rat and caffeine 18mg/kg rat intragasteric durig the same period. The fetuses were obtained from mother were decapitated and the heads were fixed in buffered formaline for at least 3 hours. The specimens were collected, prepared and examined by routine haematoxylin &eosin and immunostained by (Ki-67) marker. **Results:** at 16, 18 and 21 days of gestation, the greatest mean value was recorded in group II, followed by group III, with the least value recorded in group I. Both group I and III revealed a decrease in area percent of (Ki-67) throughout the study, whereas in group II revealed an increase. **Conclusions:** The study offers a clear evidence that lithium during pregnancy causing cleft palate in fetus and caffeine could efficiently antagonize the teratogenicity of high doses of lithium on palate when both administrated.

KEYWORDS

Lithium carbonate, Caffeine, cleft palate, Ki-67

INTRODUCTION

In pregnancy, drug treatment presents a special concern due to the threat of potential teratogenic effects of the drug [(1)]. The physiology

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of pregnancy affects the pharmacokinetics of medications used and certain medications can reach the fetus and cause harm [2]. All antipsychotics cross the placenta and, as such, consideration needs to be given to their potential to cause structural or functional dysgenesis of fetal organs and/or skeletal structures when exposure occurs in first trimester. It is generally considered that the baseline population rate for malformation in the general population is 1–3% [3]. The administration of lithium results in several congenital defects in the fetus, including cleft palate [4]. It activates Wnt/b-catenin signaling by inhibiting GSK-3b [5].

Cleft palate is a common congenital defect, affecting roughly one in 2000 newborn babies worldwide. The etiology of cleft includes multiple environmental and genetic factors [6, 7]. Lithium compounds, also known as lithium salts, are primarily used as a psychiatric medication. This includes in the treatment of major depressive disorder that does not improve following the use of other antidepressants, and bipolar disorder (ASHSP, 2015) [8]. In these disorders, it reduces the risk of suicide [9].

There are also drugs that can increase the clearance of lithium from the body, which can result in decreased lithium levels in the blood. These drugs include theophylline, caffeine, and acetazolamide. Additionally, increasing dietary sodium intake may also reduce lithium levels by prompting the kidneys to excrete more lithium [10].

A recommendation to limit caffeine intake during pregnancy was issued by the United States Food and Drug Administration in 1980. More recently, the American Congress of Obstetricians and Gynecologists reported that moderate caffeine consumption (<200 mg/day) during pregnancy does not seem to be a major risk factor of miscarriage or preterm birth [11].

The purpose of the present study was to evaluate the Biological Mechanisms of Lithium separately and in combination with Caffeine in Palatogenesis in Albino Rat.

MATERIALS AND METHODS:

Thirty six adult rats were used in this study, twenty four female and twelve male rats, after mating procedures and confirmation of pregnancy the pregnant rats were divided into 3 groups:group1(control) **,** which consists of six pregnant rats and were not receive any medication during gestation, group 2' Which consists of nine pregnant rats and were received prianil (lithium carbonate) 200mg/kg intragasteric During the period of (6th to 15th) day of gestation & group 3: Which consists of nine pregnant rats and were received prianil (lithium carbonate) 200mg/kg rat and caffeine 18mg/kg rat intragasteric durig the same period. The fetuses were obtained from mother were decapitated and the heads were fixed in buffered formaline for at least 3 hours. The collected specimens were prepared for histological and immunohistochemical studies by(ki-67)marker.

Evaluation of ki-67immunostaining by image analysis:

The number of positively reacting immunostaining of ki-67 was counted. Counting was based on the area with the highest percentage of positive cells, each stained nucleus was regarded as positive regardless of the staining intensity. The sites expressing a positive immune reaction with the ki-67 antibody are identified as brown deposits of the chromogen. The stronger the immune reaction, the darker the chromogen intensity.

RESULTS

In (**group 1**) at day 18 of gestation, The palatal shelves are completely fused and separated the oral and nasal cavities with completely disappearance of MES.The covering epithelium thins out and doesn't differentiate into stratified squamous epithelium toward the oral cavity in the midline, but in the

lateral region the epithelium begins to differentiate While in (**group 2**), At this day it was found that 5 fetuses of total 22 displayed cleft palate while the remaining 18 fetuses showed fused palatine shelves. In (**group 3**), complete fusion of palatal shelves with each other and with the nasal septum and bone trabecule formation appeared beneath nasal cavity. The epithelium covering the palate toward the OC was still thin in the midline but becomes thick at the sides (Fig. 1).

In (**group 1**) at day 21 of gestation, The palatal shelves are completely fused and separated the oral and nasal cavities with completely disappearance of MES.The covering epithelium differentiate into stratified squamous epithelium toward the oral cavity in the midline, and in the lateral region. While in (**group 2**), At this day it was found fusion of palate with the persistence of the medial epithelial seam (MES) at line of fusion, MES consists of two layer of flattened epithelial cells which represents the edge epithelium of each palatine shelves. In (**group 3**), complete fusion of palatal shelves with each other and with the nasal septum and bone trabecule formation appeared beneath nasal cavity. The epithelium covering the palate toward the OC differentiates into keratinized stratified squamous epithelium becomes thick (Fig. 1).

Both (control) and (caffeine and lithium) groups revealed a decrease in area percent of Ki-67 throughout the study, whereas the (lithium) treated group revealed an increase. Comparing the percent change in area percent of Ki-67 throughout the study in different groups, One way analysis of variance (ANOVA) test revealed that the difference of mean value was statistically significant. Tukey's post hoc test revealed no significant difference between control and caffeine groups.(Fig.1(A-F))

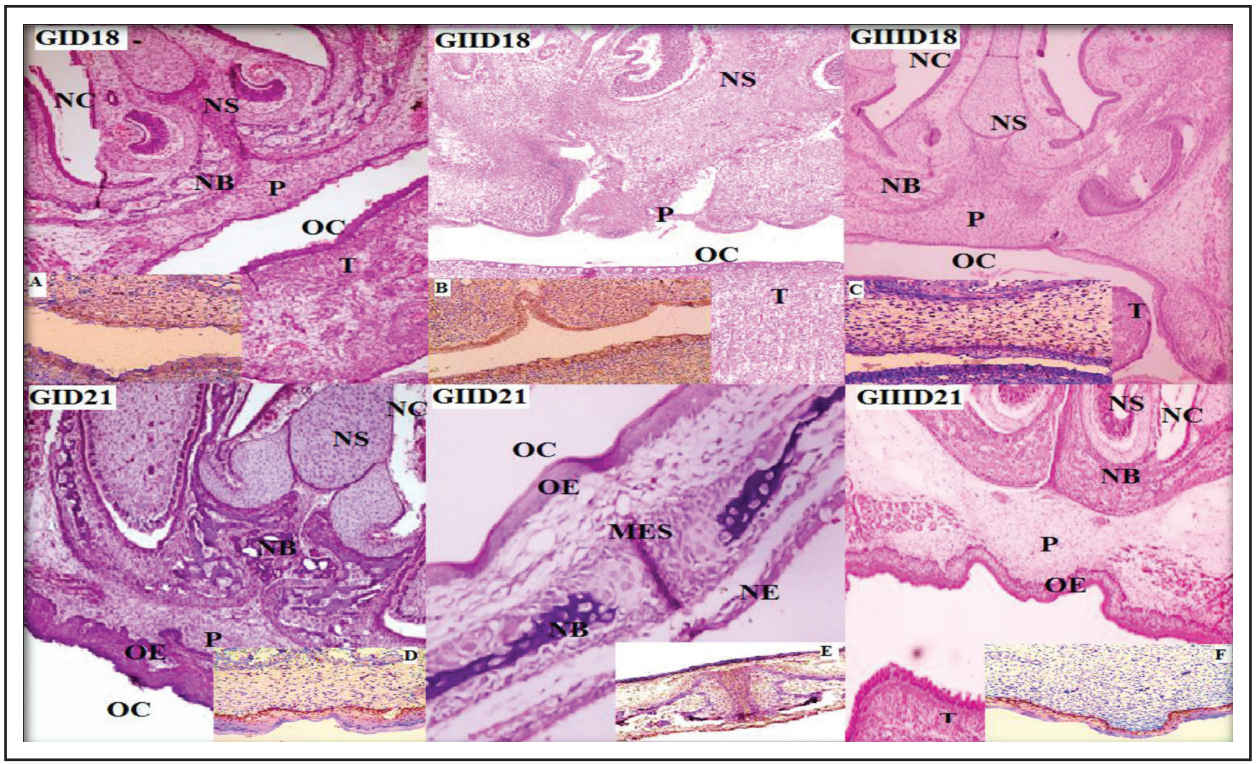

Fig. (1): GID18 (control at day 18), GIID18 (lithium group at day 18), GIIID 18 (lithium and caffeine group at day 18), GID21(control at day 21), GIID21 (lithium group at day 21), GIIID21 (lithium and caffeine group at day 21), NS(Nasal Septum), NB (Nasal Bone), P(Palate), T(Tongue), OC(Oral Cavity), NC(Nasal Cavity), OE(Oral Epithelium), NE(Nasal Epithelium).

DISCUSSION

The choice of rats in this study was according to another study [12] who reported that the early development of the human face is essentially similar to that of the rat. Also physiological body functions of rats and human are nearly similar. Besides that, it has a short period of gestation period (18- 21 days).

In this study, the average dose of choice drug (prianil C-R) for rats amounted to 200mg/kg of weight per day. This dose was calculated according to the used equation by converting adult human therapeutic dose to animal dose[13]**.** This dose resulting in cleft palate in 22.7% of fetuses accompanied by maternal mortality, miscarriage and premature labor. Similarly studies [14,15] agreed with our results.

Moreover, another study [16] reported that lithium inhibits palatal fusion and osteogenic differentiation in palatal shelves in vitro.The chosen periods of intragastric administration of 200 mg/kg prianil (lithium carbonate) to pregnant rats were from 6th to 15th day of gestation as this was the organogenesis period of pregnancy in wistar rats[17]**.**

To trace the development of palate (palatogenesis) the fetuses of the control group were sacrificed at different successive periods begin at day 14 of gestation where the development begin. Then rats were sacrificed their fetuses were tacken at days 15, 16,17 and18 of gestation to determine the position and fusion of the palatine shelves and at day 21 of gestation to determine whether the palatal formation completed or not yet. All these previous chosen periods was in accordance to others [18]**.**

In the present study the pregnant rats which received intragastric 200 mg/kg body weight of lithium at 6 to 15 days of pregnancy resulting in 22.7% of fetuses with cleft palate. These results revealed that, lithium at the present dose, period and route of administration obviously, interfere with the normal organogenesis and growth of the palatal shelves, retarded the fusion of PS through inhibition of glycogen synthase kinase 3 beta (GSK3b), resulting in persistence of MES. GSK3b plays an important role in the regulation of cell proliferation and apoptosis and the cell apoptosis is required for MES disappearance during palatal fusion. These result in agreement with others who reported that lithium inhibits palatal fusion and osteogenic differentiation in palatal shelves in vitro [16].

On the other hands, the results of various studies (including cohort, prospective, retrospective and small number case reports) indicate that lithium is a "weak" teratogen in humans and animal studies with lithium using doses comparable to human therapeutic serum levels have not reported any abnormalities[19].

To interpret the failure of PS union in this study observed at 18th and 21th days this may be due to that lithium mediated GSK3b inhibition prevents palatal fusion and osteogenic differentiation by b-catenin signaling. Disturbance of the network of signaling pathways in palatogenesis may result in a failure of fusion of the palatal shelves, and hence in a cleft palate [20].

In the present study, Lithium mediated GSK3b inhibition induced persistence of the MES, which suggests it can cause cleft . The result of this study is in agreement with previous studies in vivo and in vitro in mice [15][16]**.**Closure and fusion of the secondary palate requires timed interactions, movements, and apoptosis along the medial margins of the palatal shelves[21].

In group III which received lithium in combination with caffeine the palatal shelves were normally fused, with normal epithelial lining and without epithelial remnants in middle region. These findings revealed the enhancing effect of caffeine against lithium teratogenicity by decreasing lithium blood level through acceleration of its secretion. These results confirm the findings stated by many authors. [22,23]. On the other hand another study [24] reported that even in low doses (4 mg/day), caffeine suppresses the growth of embryonic and fetal tissues.

By utilizing antibody Ki-67 which is a nuclear protein that is associated with cellular proliferation and it is also associated with ribosomal RNA transcription (**25**).Inactivation of antigen Ki-67 leads to inhibition of ribosomal RNA synthesis **(26)**. Immunohistochemeical observation indicated that lithium carbonate increased the positive staining of ki-67 in both epithelium and connective tissues. A study (**27**) reported that there was an increase in the number of Ki-67-positive cells after administration of lithium for 14 days. another study **(28)** reported that chronic but not subacute treatment with antidepressants increase the cellular proliferation.

CONCLUSIONS:

From the present study, the following conclusions could be stated:

1. The lithium during pregnancy causing cleft palate in fetus, so during pregnancy, the benefits of medication need to be carefully weighed against risks for the mother, for the fetus, and of neonatal complications, as well as risks during breast-feeding. Accordingly, significant efforts have been made to define these risks for all medications available to pregnant women, including psychopharmacological medications
2. Caffeine could efficiently antagonize the teratogenicity of high doses of lithium on palate when both administrated in the early period of organogenesis.

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