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Research Article

Prophylactic effect of selenium against buspirone induced gestational pancreatic damage in fetuses

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Abstract

Background: Buspirone hydrochloride (Buspar) belongs to the azapirone family which is primary used to treat anxiety during pregnancy. Buspar is considered to be the safest anxiolytic drug to use during pregnancy. This work studies the effect of Buspirone hydrochloride on fetal pancreas and the postulated role of selenium to reduce the adverse effects of the drug. **Aim of the work:** The aim of this work is to investigate the destructive effect of buspirone on the fetus pancreas and the possible antagonistic effect of selenium. **Material and methods:** buspirone hydrochloride tablets were obtained from Beecham pharmaceutical, Cairo, Egypt. Eighty-one albino rats were used throughout the study, 54 female and 27 males. The rats were assigned into three groups eighteen female albino rats with nine male albino rats. Group I: In this group, received water only. Group II: In this group, pregnant rats were given oral doses of buspirone at a dosage of 4.1mg/kg/day, from the 6th day to the 20th day of pregnancy. Group III: In this group, the pregnant rats were given oral buspirone at a dose of 4.1 mg/kg/day from the 6th to the 20th day of conception, with oral selenium at a dosage of 0.3mg/kg/day. Fetuses were collected and the pancreases were harvested for histological, and immunohistochemical analyses. **Results:** Buspirone induced marked histopathological changes fetal pancreases which was remarkably ameliorated by the prophylactic use of Selenium. **Conclusion:** This work revealed a prophylactic role for selenium in uspirone induced fetal pancreatic damage. Thus, selenium administration to patients on buspirone is recommended during pregnancies to avoid offspring pancreatic damage

Keywords: Buspar, Selenium, Fetus, Pancreas

Introduction

Pregnancy has an effect on psychological state of pregnant women, with an increase in anxiety and discomfort[1]. Psychotropic drugs prescription is limited during pregnancy due to the potential risks risks on offspring's. However, stopping medication exhibits new dangers [2].

Buspirone is a strong anti-anxiety drug. It works on the serotonin 5-HT_{1A} receptors. Clinically, buspirone is not accompanied by adverse effects of sedation, addiction, or cognitive impairment [3].

Selenium has antioxidant roles. The thioredoxin reductase group of selenium has numerous biochemical reactions such as thiol redox regulation, and DNA synthesis [4].

In this study, we aim to investigate the effect of Buspirone administration during pregnancy on the pancreas of fetuses and the possible protective influence of selenium administration along with the drug

Material and Method

Buspar as pills From Beecham pharmaceutical, Cairo, Egypt. It was given orally by the nasogastric tube as a single oral doses daily 4.1 mg /kg/day[5], to the buspirone group**.** Selenium (Sigma-Aldrich, Cairo, Egypt) was co-administered with buspar we orally by nasogastric tube at a dose of 0.3mg/kg/day[6]**.**

Animals and treatments:

The Ethical Committee of Minia University (Approval no. 691:11/2020) gave its consent to the experiment. Fifty-four adult female albino rats and 27 adult male albino rats we brought them from the Minia University, Laboratory Animals Unit, and were maintained in appropriate to be mated. Their weights were between 200-250g. The animals were divided into three groups each 18 female albino rats with 9 male albino rats. We searched for the plug in the vagina every day. The first day of pregnancy considered when vaginal plug was seen. Pregnant animals were treated from 6^{th} day until 20^{th} day and assigned into three groups: 18^{th} pregnant rats in each group

Group I (control): received refined water equivalent to the dose of the drug given to other groups.

Group II (Buspar): Buspirone hydrochloride 4.1 mg/kg/day was given orally by nasogastric tube[5].

Group III (Buspar-Se): Buspar along with selenium were given, selenium was given orally by nasogastric tube at a dosage of 0.4 mg/kg/day[6]**.**

Histological Study: At the end of the experiment, the pregnant rats were anesthetized, then the fetuses were obtained and fetal pancreases were harvested and, and cut apart to obtaine the pancreas which immersed in ice-cold 0.15 ml NaCl and dried by filter paper, then it was preserved in formalin, a buffered isotonic solution of 37% formaldehyde. Pancreases were then dried out by ascending concentration of alcohol, then washed with xylol. Samples were processed for paraffin embedding. Paraffin blocks were sectioned and stained by hematoxylin and eosin for histopathological analysis.

Immunohistochemically study (caspase-3): In brief, paraffin-immersed fetal pancreas was treated with 0.3% hydrogen peroxide in Phosphate-Buffered Saline (PBS) for 30 minutes to prevent the endogenous peroxidase. In the primary anti-rat antibody against caspase (cysteine-aspartic proteases) we put the parts of pancreas. The tissues were then bathed three times in PBS before being kept for 1 hour at 20 to 25°C with goat anti-rat peroxidase-combined secondary antibody. The immune reaction was detected using chromogen 3,3′diaminobenzidine hydrogen peroxide, then parts were stained with hematoxylin.

Method for morphometric analysis :the photos of slides enter into imag j programme to estimate the discolored area and its density.

Results

Pancreatic tissues stained with in Hematoxylin and Eosin (H&E):

Group I (Control group): fetal pancreases showed normal architecture of pancreatic acini and duct system. Acinus cells were seen cuboidal in shape and contained eosinophilic cytoplasm and basal active basophilic nucleus. (Fig. 1-A)

Group II (Buspar group): The fetal pancreases showed less developed pancreatic tissue fetuses. Many of acini exhibited failure of development, deep stained basophilic nucleus in addition to necrotic changes in numerous cells. (Fig. 1-B)

Group III (Buspar – selenium group): Pancreas showed a a variable degree of histopathological changes, some normal pancreatic tissues with normal acini and nucleus. There was some acini revealed failure of the development, and a deep basophilic nucleus. Occasionally degenerative and necrosis in some cells. (Fig. 1-C)

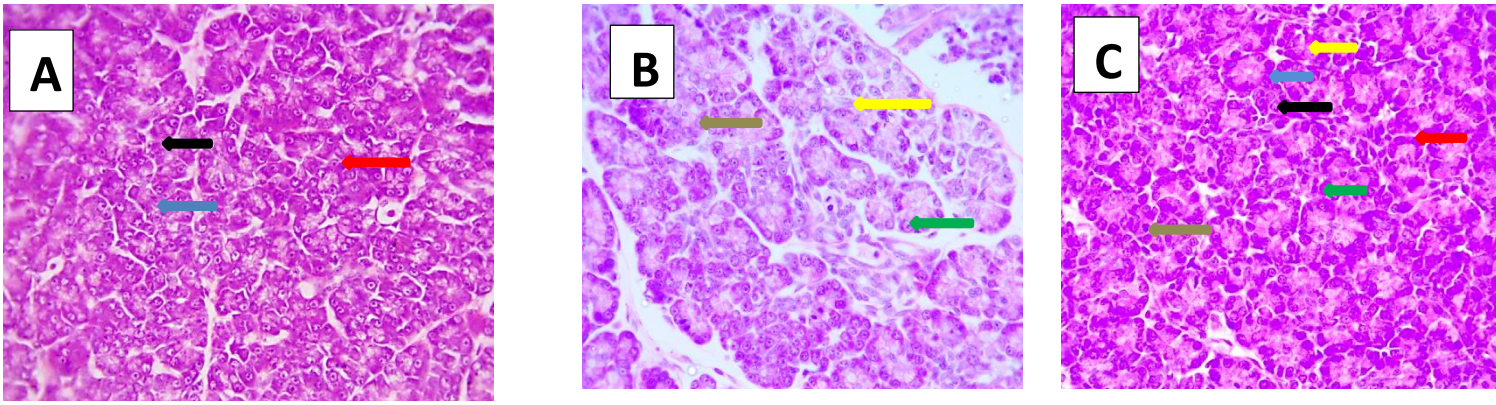


Fig. 1 (A, B, C):): photographs of parts of the fetal pancreas stained with Hematoxylin and Eosin of control group (A), Buspar group (B), and Buspar-selenium (C). Showing ordinary structure of acini (blue arrow), ordinary duct (red arrow), and active nuclei (black arrow) in figure A and C. in the figure B and C, there are deformed acini (green arrow), highly stained nonfunctioning nuclei (gray arrow), and necrotic material (yellow arrow), H&E X400

Pancreatic tissues stained with Periodic Acid Schiff (PAS):

1-Group I (Control group): carbohydrates were found plentiful in the fetal pancreas, with habitual magenta-colored elements united in the cytoplasm of cells of pancreas. (Fig. 2-A)

2-Group II (buspirone group): a marked decline in the carbohydrate particles in relation to the control one, faint magenta-colored elements were observed. (Fig. 2-B)

3-Group III (buspirone – selenium group): modest reduction in the carbohydrate particles in relation to the control one, modest magenta-colored elements were observed. (Fig. 2-C)

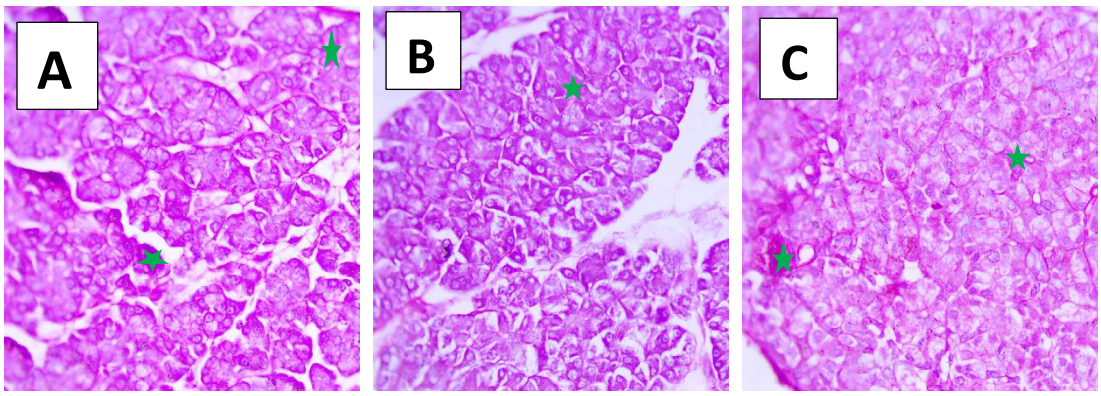


Fig. 2 (A, B, C): Photographs of regions of the pancreas of fetus discolored with Periodic Acid Schiff of control mother (A), treated with Buspar (B), and treated with Buspar-selenium (C). Revealing ordinary quantity of magenta-colored elements which signify the carbohydrates (green star) in control group (A), reduction magenta-colored elements in Buspar group (B), and modest of magenta-colored elements in Buspar-selenium group (C), PAS X400

B) Immunohistochemically (caspase-3):

1-Group I (Control group):): A limited number of pancreatic acini were weakly stained with caspase-3, the brown staining concentration was less. (Fig. 3-A)

2-Group II (Buspar group): several pancreatic acini were intensely stained for the caspase-3 (Fig. 3-B)

3-Group III (Buspar – selenium group): A moderate areas of the pancreatic acini cells were stained by the caspase-3. (Fig. 3-C)

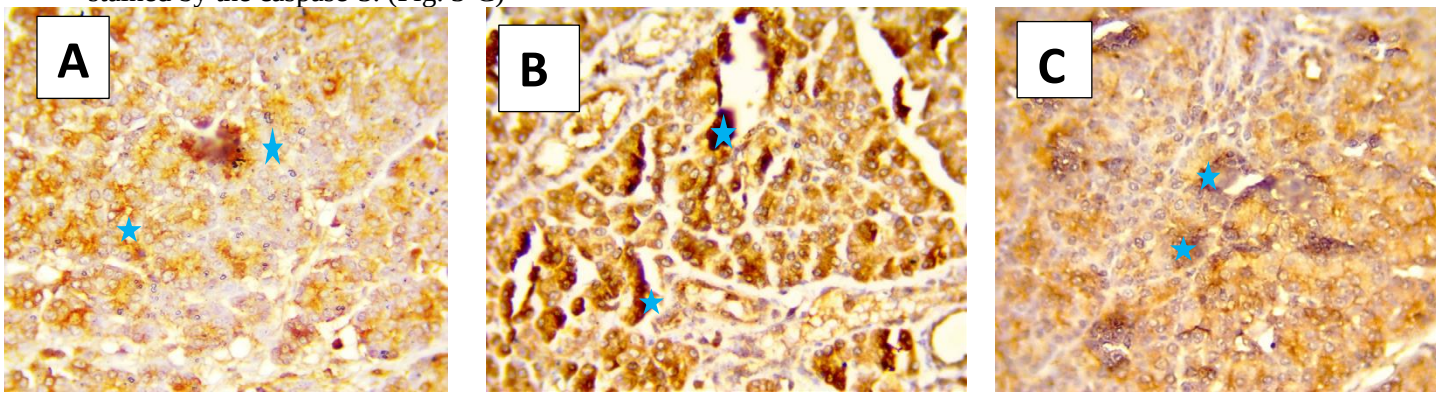


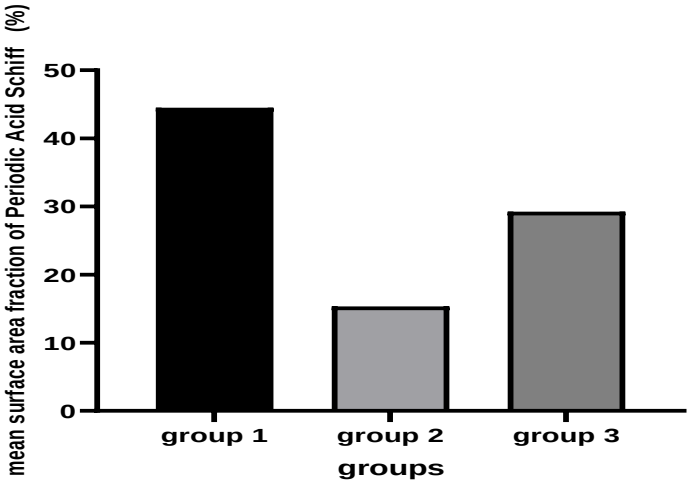
Fig. 3 (A, B, C): Photos of segments of the pancreases of fetus stained with caspase-3 of control mother (A), treated with Buspar (B), and treated with Buspar-selenium (C). Display ordinary range of apoptosis weak brown color (blue star) in control group (A), increase density of apoptosis deep brown in buspirone group (B) and modest quantity of apoptosis fair brown color in Buspar-selenium group (C), caspase-3 X400

Statistical analysis:

The mean surface fraction area ± standard deviation (SD) was used to compare the Periodic Acid Schiff, and the caspase-3 positive ingredients substances in the three groups by One Way ANOVA test.

Table 1: displaying the mean surface area fraction amounts of PAS-positive Discolored are in the pancreas of the fetus in the control, Buspar, and Buspar-Se groups [7], [8].

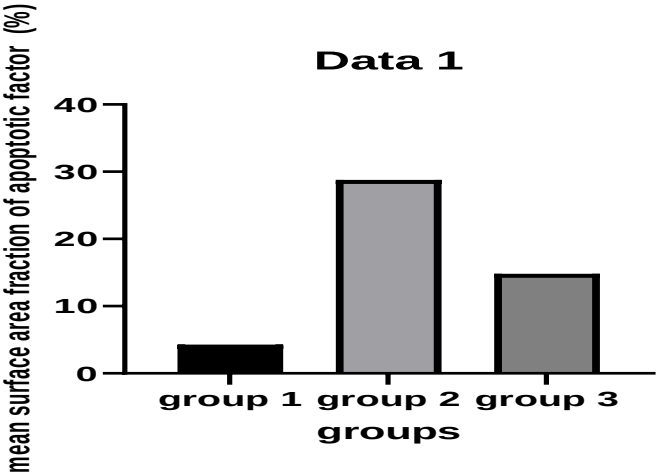
Group / Parameter	Group 1 (Control)	Group 2 (Buspar)	Group 3 (Buspar- Se)
mean ± SD	44.50±4.309	15.33±0.9866	29.23±2.957
P- value		*0.0003	*0.0072 **0.0015



- The results are significant at *P ≤ 0.05, * comparison with control, ** comparison with Buspar.
- Bar chart representing the carbohydrates amount between different groups. A noticeable reduction in the amount of carbohydrates particles existing in the Buspar group,

Table 2: displaying the mean surface area fraction amounts of caspase-3 positive discolored in the pancreas of fetus of the control, Buspar, and Buspar-Se groups [9], [10].

Group / Parameter	Group 1 (control)	Group 2 (Buspar)	Group 3 (Buspar-Se)
mean ± SD	4.267±1.801	28.77±3.691	14.80±1.247
P-value		*0.0005	*0.0011 **0.0034



- The results are significant at *P ≤ 0.05, * comparison with control, ** comparison with Buspar.
- Bar chart illustrates the comparison of caspase-3 between the three groups. The prominent range of apoptosis exhibits in the Buspar group,

Discussion

Anxiety and stress during pregnancy are linked with spontaneous abortion, preterm delivery, fetal malformation, and delivery difficulties[2]. Buspirone is an effective anxiolytic medication [3]. It is classified as group B in pregnancy, which means that it is classified as safe to use in pregnancy [11]. In the research of Zhao, X., et al., study [12], declared that strict selenium insufficiency, caused atrophy of the pancreas and poor growth.

The development of exocrine part of the fetus of rats is noticed to be functioned by Direnzo, D., et al.,[13], at almost the age of 19th day of intrauterine life in the form of elaborated zymogens granules.

In our study, we conducted to assess the effect the usage of Buspar while women is pregnant on the fetus pancreas and the shielding effect of selenium to improve the damage produced by buspirone.

In the Hematoxylin and Eosin staining, the findings were the same as El-Shaer et al.,[14] in the study of control group, it exhibited normal structure of acini and ducts with active nucleus and in the

Buspar group, many acini exhibited failure of formation of pancreatic tissues, uniform strong eosinophilic cytoplasmic ingredients, as well as necrosis of many cells. The similar damage of the pancreas was also found in Zhao et al., study [12], in the form of pancreatic deterioration due to lack of selenium.

Buspar had been noticed to influence other organs. In the kidneys, Buspar displayed degeneration in the kidney tissues in the form of destruction of proximal and distal convoluted tubules[15]. Also, in the cerebellum, there were obvious deterioration in the cerebellar tissues in the form of deformity in the granular and Purkinje cells layer[5].

Depletion of carbohydrates in the cells of the fetus organs due to Buspar was documented in El-Shaer et al., study, in the pancreatic cells compared to control group agreed with same findings we found[14], in the cells of kidneys[15], and in the cerebeller cells[5]. The partial restoration of the normal amounts of polysaccharides stained by PAS in the buspirone-selenium group was decum-ented that the selenium regulated carbohydrate enzymes activities agreed with the same findings we found in third group[16].

In the immunohistochemical study, we found observable reaction of caspase-3 in the control group which was unlike the El-Shaer et al., finding[14], who stated that sections were negatively stained with caspase-3. Existence of little proportion of programmed cell death in the living tissues was recorded by Jakubowska et al., agreed with our findings[17], also documented that in the control group existed a low percentage of active caspase-3 agreed with our finding[10]. Programmed cell death in control pancreatic tissue ranges from 3% to 7%,[18]. Apoptotic level elevates in drugs that induces destruction in the pancreas agreed with our finding [9]. Some of the malformations caused by the buspirone in the fetus of the albino rats were documented in the form of some fetuses were born died, and decrease in the weight of fetuses[19].

Conclusion

Giving buspirone during pregnancy affected the development of the fetal pancreas in the form of distorted acini, decreased its activity in the form of depletion of polysaccharides particles and these changes reduced with adding selenium.

Recommendations

Administration of buspirone during pregnancy should be for short period, and the lowest dose. Further studies on the

effect of buspirone administration during pregnancy on the organogenesis of the fetus is strongly required.

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