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Research Article
Histopathological, Histochemical and Immunological Studies on Fetal Pancreatic Tissue of Rats Treated with Carisoprodol

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Abstract

Background and Objective: Carisoprodol is a musculoskeletal relaxant associated with painful musculoskeletal conditions and there are no adequate studies concerning Carisoprodol effects on fetuses treated maternally with Carisoprodol. This study was conducted to clarify the histopathological, histochemical and immunological changes in fetal pancreas of Albino rats after maternal treatment with high and low doses of Carisoprodol drug (Somadril). **Materials and Methods:** Thirty adult pregnant rats were used in the present work and categorized into three groups (ten pregnant female rats in every group) first group served as the control group (C). The second and third groups (S1 and S2) were treated with oral doses of Carisoprodol in the distilled water equal to 10.8 and 21.6 mg/100 g, respectively of body weight/day. Groups S1 and S2 were administered for 15 days from day 6 to the 20th days of pregnancy. Pregnant rats were sacrificed on the 20th day of pregnancy and small sections of fetal pancreatic tissue were reserved for histopathological, histochemical and immunological studies. **Results:** Numerous histopathlogical, histochemical and immunological alternations were detected in the fetal pancreatic tissue of the two groups after maternal treatment with high and low doses of Carisoprodol compared to the control group. **Conclusion:** The present study showed that administration of Carisoprodol caused several histological, histochemical and immunohistochemical deformity in the fetal pancreatic tissues.

Key words: Carisoprodol, fetuses, pancreas, histopathology, histochemistry and immunology

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Skeletal muscle relaxants have a variety of structurally not linked structures that can be separated into 2 portions: antispasmodic and antispasticity factors. These medications have various uses, side-effect outline and mechanisms of action. And spasticity factors work on the spinal cord or immediately on the skeletal muscles to progress muscle hyper tonicity and automatic convulsion while, antispasmodics drugs lowering muscle spasms by changes of CNS conduction[1]. They are branched into benzodiazepines, (e.g., diazepam) which inhibit transition on the postsynaptic γ-aminobutyric acid (GABA) neurons and are utilized as sedatives, anxiolytics and anticonvulsants, also have been shown to have efficiency in the severe management of low back pain[2]. Other types of antispasmodics are non benzodiazepine agents which act at the brain stem and spinal cord[1]. Carisoprodol (Soma compound) is widely prescribed skeletal muscle relaxant specified for the intense treatment of musculoskeletal pain conditions[3]. Carisoprodol drug is merchandise under an assortment of trademark names (Soma, Somadril, Somalgit, Somacid, Vanadam, Carisoma, Relaxibys, Sonoma, Scutamil C, Mio Relax, , Rela and Soridol)[4]. Soma compound is a chemical with a mild characteristic odor, a neutral white crystalline powder with a slightly bitter taste; it is an aliphatic dicarbamate and a chemical derivative of meprobamate[5]. This drug is soluble easily in water, but insoluble in vegetable oils[6]. Carisoprodol is available as a prescription drug in 250 mg and 350 mg tablets; both doses are generally well tolerated, but 250 mg is similarly active and well stood than Carisoprodol (350 mg)[7]. Carisoprodol (Soma) compound leads to the central nervous system depression with reduction in perception of pain[5]. The same authors added that Carisoprodol effects on the central nervous system (CNS) may include drowsiness, dizziness, ataxia, tremor, blurred vision and headache. In 2000, In the US, Carisoprodol was classified as the second utmost commonly prescribed muscle relaxant[8]. The specific technique of action of drug is obscure, but it is associated with inhibiting inter neuronal movement and dejection of the polysynaptic neuronal transmission in the reticular formation and the spinal cord[9]. Once ingested, Carisoprodol drug is metabolized by the liver into three primary metabolites (hydroxyl Carisoprodol, hydroxyl meprobamate and meprobamate)[10] and defecated by kidneys, this drug must be used by special care in the patients with weakened renal or hepatic functions[11]. Carisoprodol may stimulate GABAA receptors independent of the effect of meprobamate[9] which is a sedative-hypnotic that is commonly used in treatment of anxiety. The central action of meprobamate effects on GABAA receptors (GABAARs) by barbiturate like stimulating activity at GABAA receptors and the predominant inhibitory neurotransmitter receptor in the brain[12]. Effects of Carisoprodol of both the sedative and adverse are due to its metabolic conversion to meprobamate[13]. The pancreas is a remarkable organ it synthesizes and secretes enzymes engaged in the digestion[14]. It gives sundry hormones; as glucagon and insulin that adjust blood sugar levels[15]. A previous animal study indicated that Carisoprodol treatment of the pregnant rats crossed the placenta and resulted in adverse effects on the fetal growth and postnatal survival, as severe variations in the esophageal tissue of fetuses[16]; maternal and fetal liver tissue[17]; histopathological, histochemical and immunological changes in maternal and fetal lung tissues[18] and kidney cortex[19]. There is no obtained information about the effects of Soma drug treatment on the fetal pancreatic tissue treated maternally with Carisoprodol so; this study aimed to evaluate the histopathological, histochemical and immunological changes of fetal pancreas due to Carisoprodol with two different doses which administration in pregnant lab rats.

MATERIALS AND METHODS

Study area: This work was carried out at Zoology Department, Faculty of Science, Al-Azhar University, Egypt from June-August, 2018.

Drug: Carisoprodol drug (Somadril) is composed of tablets which are combination of products containing Carisoprodol 200 mg, 160 mg paracetamol and 32 mg caffeine and was purchased from Mina Pharm, Pharmaceuticals and Chemical Industries, Cairo, A.R.E. The drug is used for the relief of disturbance linked by severe, tender in musculoskeletal statuses in mature.

Experimental animals: In the present study, adult Sprague Dawley male and female rats, weighing (150-200 g) were used received from the animal house of El-Nasr Pharmaceutical Chemicals Company, before beginning the experimental study. Daily, all female rats were examined through vaginal smear to detect the estrous phase. They were reproduced with 2 females caged overnight with a single male. In the morning, a vaginal smear was performed for checking the existence of sperms. Presence of sperms or vaginal plug-in vagina was considered that mating was occurred and that indicated the 0 day of gestation[20]. Thirty adult pregnant rats were used in this work and classified into three groups (ten gravid rats in each set), the 1st set was served as control group (C). The 2nd

group (S1) was treated with oral dose of Carisoprodol in the distilled water equivalent to 10.8 mg/100 g body weight/day. The third group (S2) gravid rats were preserved with oral dose of the Carisoprodol identical to 21.6 mg/100 g body weight/day. Groups S1 and S2 were administered for fifteen days from day 6 to day 20 of gravidity. Equal dose for the pregnant rats was calculated as Paget and Barnes[21].

Histopathological investigations: The gravid rats were sacrificed on 20th day of pregnancy and small pieces of fetal pancreas were picked up and fixed in Bouin's solution and neutral buffer formol for 24 hrs and prepared for paraffin serial sections (5 μm (for the histological study and stained with hematoxylin and eosin H and E[22]; Mallory's trichrome technique for detecting collagen fibers[23].

Histochemical investigations: Histochemical stains were established for determination of polysaccharides by applying Periodic acid Schiff's (PAS) reagent[24]; mercuric bromophenol blue to determine the content of protein[25]. Feulgen reaction to detect the DNA materials[26], Congo red stain to detect the amyloid-β protein[27].

Immunohistochemical investigations: Neutral buffered formalin (10%) fixed pancreas sections (5 μ thick), paraffin-embedded fetal tissues were de-waxed, hydrated to phosphate-buffered saline (PBS; pH 7.5) for 5 minutes and then incubated with an endogenous peroxidase blocking reagent. For immuno-staining pretreatment of slides was done by boiling for 10 min in 10 Mm citrate buffer at pH 6 for antigen retrieval and cooling sections at room temperature for 20 min. Then, sections were incubated overnight horizontally in a humid chamber at room temperature for 60 min with one to two drops of the supersensitive primary antibody [caspase 3 and insulin] were then they were put on the sections. The sections were rinsed for 5 min in PBS and incubated with goat anti-rat peroxidase-conjugated secondary antibody (peroxidase-labeled streptavidin) for 1hr at room temperature and rinsed again three times in PBS. The immunoreactivity was visualized using DAB 3,3-diaminobenzidine hydrogen peroxide as a chromogen and the whole procedure was finished then nuclear counter staining was done using Mayer's hematoxylin (Hx)[28].

RESULTS

Histopathological examinations: The pancreatic tissue of C group discovered early-developed pancreatic islets of Langerhans randomly distributed among the acinar structures. Their number, size and distributions were variable and few α-cells were seen with indistinct location within the islets (Fig. 1a-b). Fetal sections of S1 group pointed out pancreatic structures formed mostly from the exocrine acinar and ductal structures with ill-distinct endocrine counterpart, ill-distinct cell boundaries with nearly complete disappearance of the nuclei with presence of some pyknotic ones, highly overfilled blood pots which contained hemolyzed blood cells and highly widened stroma in between the pancreatic acini (Fig. 1c-d). Examined fetal sections of S2 group revealed well organized, healthy pancreatic structures with presence of moderate number of islets of Langerhans randomly distributed among well-formed pancreatic acini (Fig. 1e-f). Examination of Mallory's trichome stained sections in the pancreatic sections of C group showed thin distinct amount of collagen fibers (Fig. 2a). Fetal sections from pancreas of group S1 revealed amplified collagen in the cytoplasm of pancreatic cells of fetuses, pancreatic ducts and islets of Langerhans (Fig. 2b). In the group S2 (Fig. 2c), there is highly amplified collagen packages were detected around and inside the exocrine part, stroma in between them and islets of Langerhans.

Histochemical investigations: Pancreas of C group displayed soberly PAS+ve materials in the pancreatic acini, pancreatic islet of Langerhans. Increased polysaccharides the pancreatic acini, pancreatic islets of Langerhans were seen in group S1. Highly enlarged PAS+ve materials were detected in pancreatic acini, pancreatic islets of Langerhans of group S2. Moderately to intensely stained protein contents were noted in the cytoplasm of fetal pancreatic cells with less staining affinity in the stroma in between them. S1 and S2 groups reported deeply increased staining affinity of total protein in the cytoplasm of fetal pancreatic cells and in the blood vessels. Amyloid-β protein were faintly stained in cytoplasm of the fetal pancreatic cells and stroma in between them in C group. Highly amplified amyloid- β protein in cytoplasm of pancreatic cells, blood vessels, stroma in between them, pancreatic duct and islets of Langerhans in S1 group. Highly increased amyloid-β protein was realized in and around the exocrine parts, stroma in between them and islets of Langerhans of S2 group. Moderately stained red purple-colored DNA materials were observed in cytoplasm of the control fetal pancreatic cells and islets of Langerhans. In fetuses maternally treated with the low dose (S1 group) the nuclei were markedly shrunk with decreased DNA materials in the pancreatic acini and pancreatic islets of Langerhans, but in group S2 the DNA-containing bodies acquired reddish violet stained granular elements in the pancreatic acini and pancreatic islets of Langerhans.

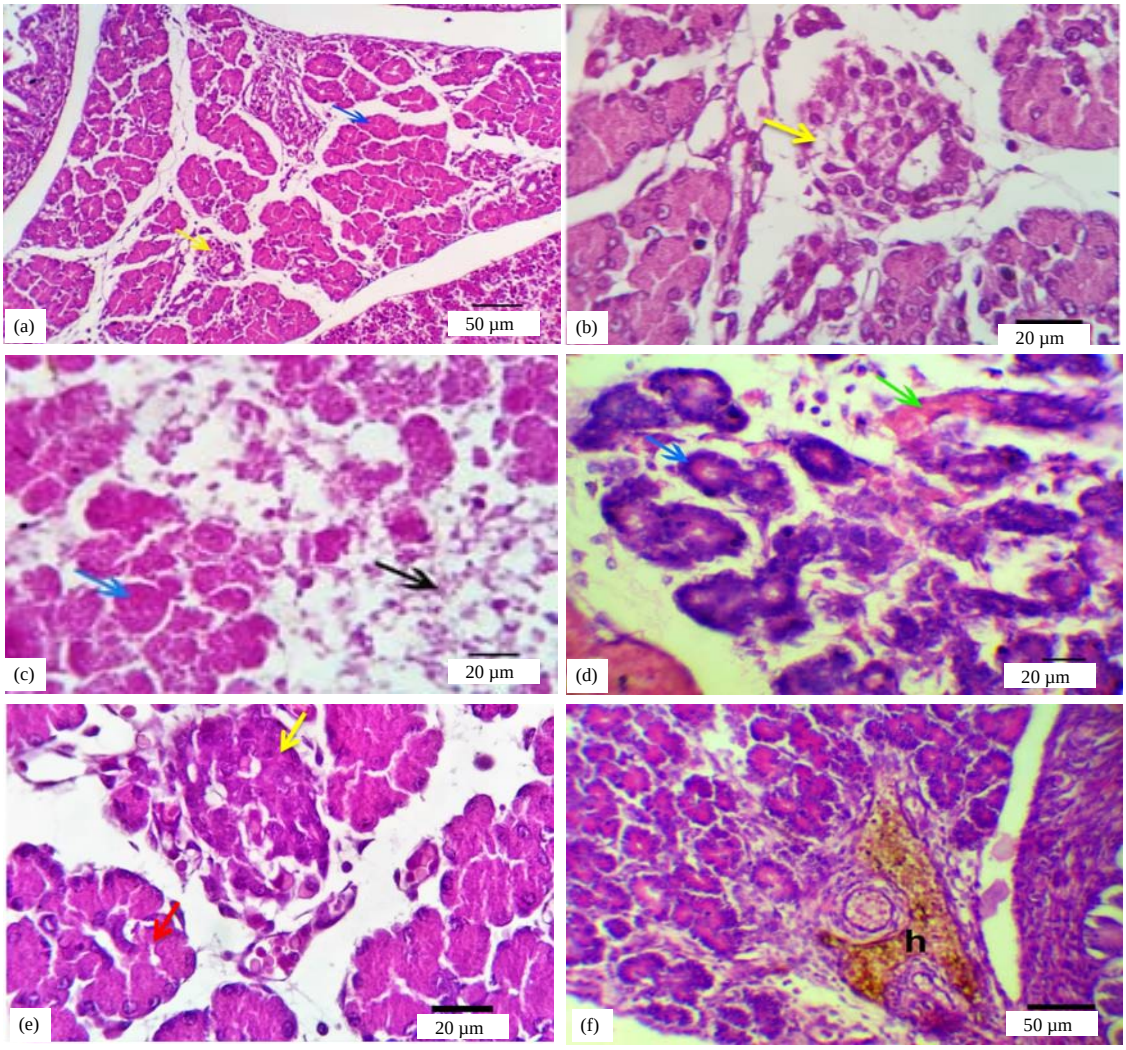

Fig. 1(a-f): Photomicrographs of fetal pancreas sections stained with H and E. (a-b) Control group show early developed pancreatic islets of Langerhans (yellow arrows) distributed among the acinar structures (blue arrow). (c-d) S1 group show pancreatic structures formed mostly from the exocrine acinar and ductal structures with ill-distinct endocrine counterpart, ill-distinct cell boundaries with nearly complete disappearance of the nuclei and or presence of some pyknotic ones (blue arrows), highly crammed blood vessels which contain hemolysed blood cells (green arrow), highly widened stroma in between the pancreatic acini (black arrows). (e-f) S2 group show well organized, healthy pancreatic structures with presence of islet of Langerhans (yellow arrow) randomly distributed among well-formed pancreatic acini (red arrow), but, highly dilated and congested blood vessel is detected; it contains numerous hemolysed RBCs surrounded by large hemorrhagic area (h)
Scale bars 50 µm in A and F and 20 µm in B, C, D and E

Immuno-histochemical investigations: Sections from immuno-stained fetal pancreatic tissue of the control group showed primitive acini and islets of Langerhans (Fig. 7a). Examined sections of group S1 revealed primitive acini and islets of Langerhans with highly condensed stain for the apoptotic marker Caspase 3 (Fig. 7b). Group S2 showed a primitive acini and islets of Langerhans with very highly condensed staining affinity for the apoptotic marker Caspase 3 (Fig. 7c). Examined sections from immuno-stained fetal pancreatic tissue of the control group denoted a moderate number of randomly distributes islets of Langerhans with a positive staining reactivity of β-cells to the anti-insulin monoclonal antibody (insulin-marker). About 80-90% of the β cells appeared positively stained with the developer

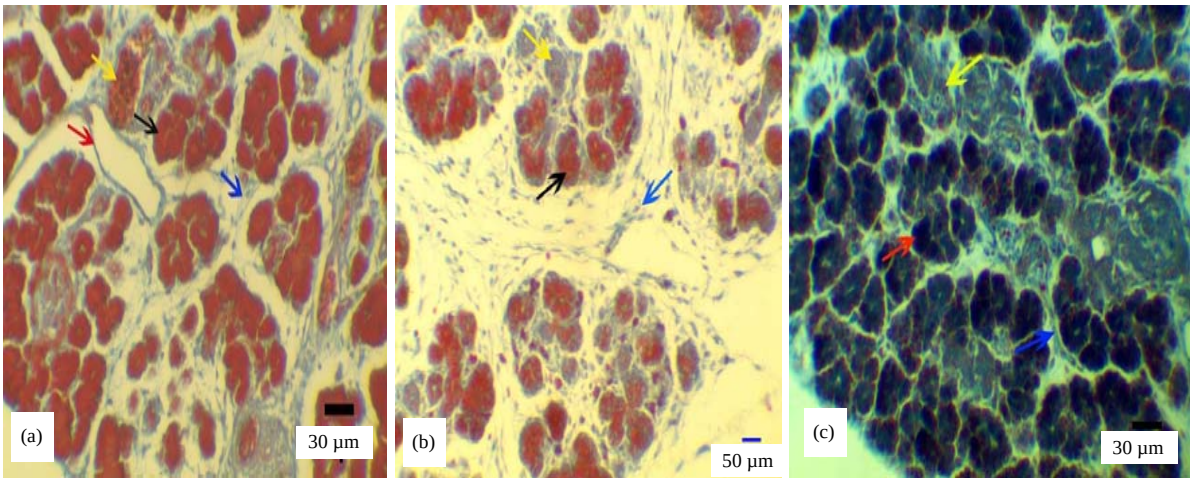

Fig. 2(a-c): Photomicrographs of fetal pancreas sections stained with Mallory's trichome stain. (a) Control group show distinct amount of collagen fibers in the pancreatic acini (black arrow), pancreatic islet of Langerhans (yellow arrow), in wall of the blood vessel (red arrow) and normal stroma (blue arrow), (b) S1 group shows increased collagen fibers in the cytoplasm of fetal pancreatic cells (black arrows), pancreatic duct (blue arrow) and islets of Langerhans (yellow arrow) and (c) Group S2 shows extremely amplified collagen bundles around and in the exocrine part (red arrow), stroma in between them (blue arrow) and islets of Langerhans (yellow arrow)
Scale bars 50 µm in B and 30 µm in A and C

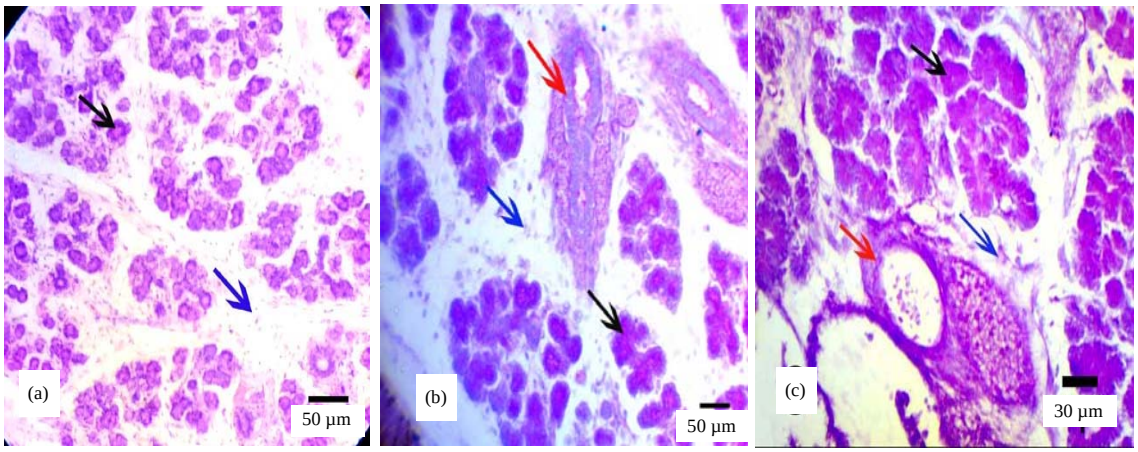

Fig. 3(a-c): Photomicrographs of fetal pancreas sections stained with Fulgen reaction. (a) C group shows moderately stained PAS+ ve materials in the cytoplasm of fetal pancreatic cells (black arrows) and poorly stained stroma in between them (blue arrow) and (b-c) S1 and S2 groups show highly amplified PAS+ve materials in the cytoplasm of fetal pancreatic cells (black arrows) and poorly stained stroma in between them (blue arrow) and walls of blood vessels (red arrow)
Scale bars 50 µm in A and B and 30 µm in C

chromogen conjugate as cytoplasmic fine brownish granular materials (Fig. 8a-b). Sections from the immuno-stained fetal pancreatic tissue of S1 group revealed a few number of islets of Langerhans of relative small to moderate sizes randomly distributed among the acinar structures with about 60-70% staining reaction to the insulin bio-marker (Fig. 8c-d). Group S2 pointed out a relatively moderate to numerous numbers and sizes of randomly distributed islets of Langerhans with a positive staining reactivity of β cells. About, 80-90% of β cells appeared positively stained as cytoplasmic fine brownish granular materials (Fig. 8e-f).

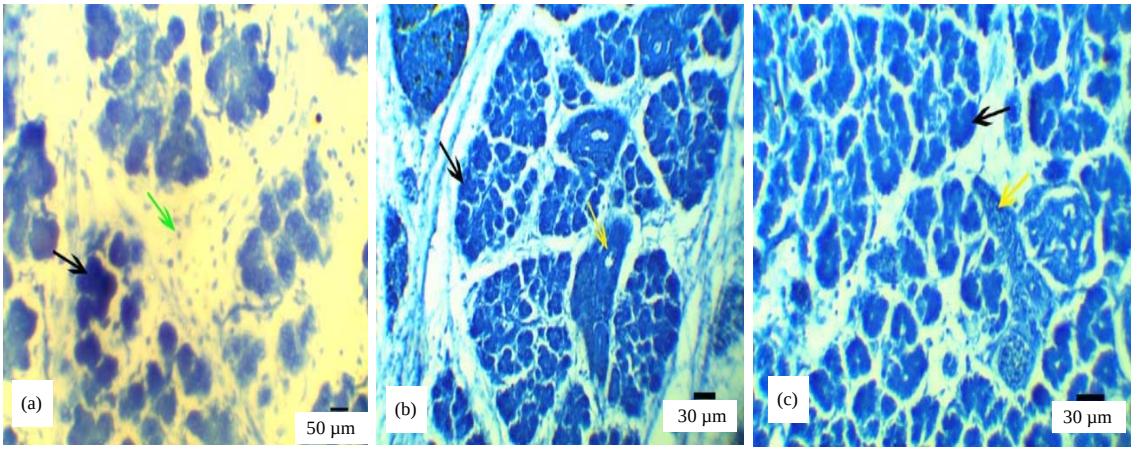

Fig. 4(a-c): Photomicrographs of fetal pancreas sections stained with Mercuric bromophenol stain. (a) Group of control showing moderately to deeply stained protein materials in the cytoplasm of fetal pancreatic cells (Black arrow) with less staining affinity in the stroma in between them (Green arrow), (b) S1 group increased staining affinity of total protein content in pancreatic cells (Black arrow) and in blood vessels (yellow arrow) and (c) S2 group showing highly increased staining affinity of total protein in the cytoplasm of fetal pancreatic cells (Black arrow) and in islets of Langerhans(yellow arrow)
Scale bars 50 µm in A, 30 µm in B and C

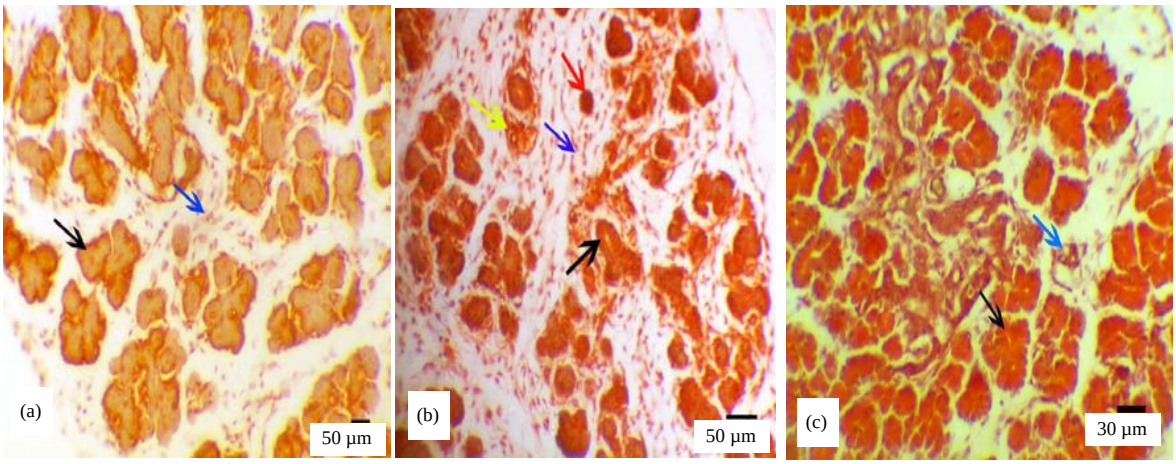

Fig. 5(a-c): Photomicrographs of fetal pancreas sections stained with Congo red stain. (a) Set of control shows faintly stained amyloid-β protein in the cytoplasm of fetal pancreatic cells (black arrow) and stroma in between them (blue arrow), (b) S1 group shows highly increased of amyloid-β protein in the cytoplasm of fetal pancreatic cells (black arrow), blood vessels (red arrow), stroma in between them; pancreatic duct (blue arrow) and islets of Langerhans(yellow arrow) and (c) S2 group) shows highly increased amyloid-β protein in and around the exocrine part (black arrows); stroma in between them (blue arrows) and islets of Langerhans
Scale bars 50 µm in A and B, 30 µm in C

DISCUSSION

The current study clarified histopathological, histochemical and immunological alternations in the fetal pancreatic tissue of pregnant rats treated with Carisoprodol.

The pancreas was a highly important organ for life of vertebrates as it plays a central role in digestion and regulates blood sugar[29]. In animals, carisoprodol was thought to performance centrally by producing sedation as an alternative of direct skeletal muscle relaxation by stopping interneuron

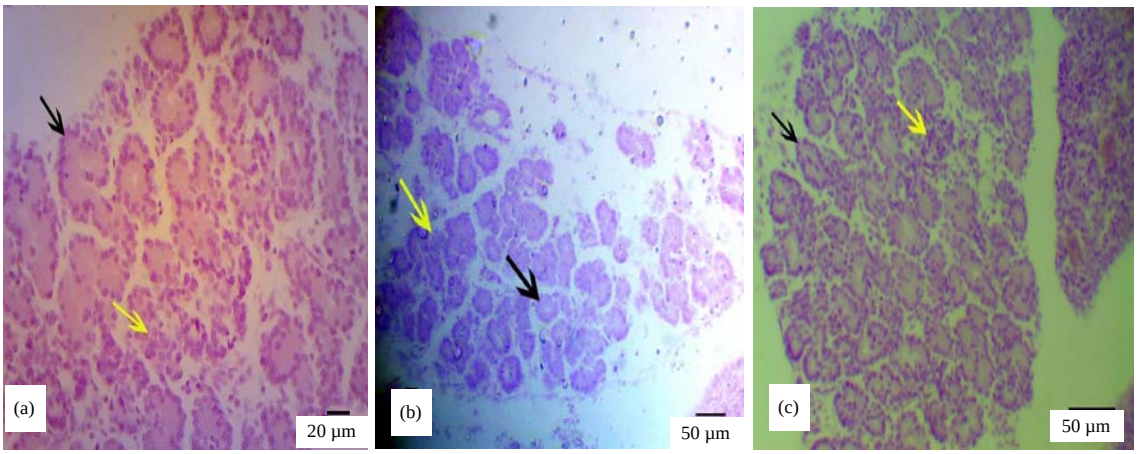

Fig. 6(a-c): Photomicrographs show DNA materials in the fetal pancreas sections. (a) Group of control shows moderately stained DNA materials in the pancreatic acini (black arrow), pancreatic islet of Langerhans (yellow arrow), (b) S1 group showing shrunk nuclei with decreased of DNA materials in the pancreatic acini (black arrow), pancreatic islet of Langerhans (yellow arrow) and (c) S2 group showing a nuclei appeared packed with intensely stained DNA materials in the pancreatic acini (black arrow), pancreatic islet of Langerhans (yellow arrow)
Scale bars 20 µm in A, 50 µm in B and C

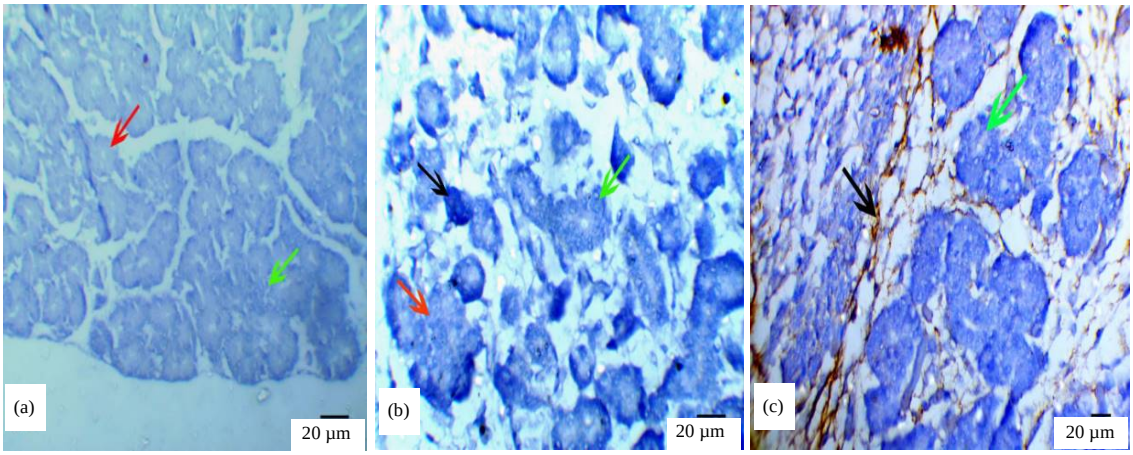

Fig. 7(a-c): Photomicrographs of fetal pancreas sections stained with caspase 3. (a) Control group shows primitive acini (red arrow) and the islets cells (green arrow) negatively stained for the apoptotic marker caspase 3 (black arrow), (b) S1 group shows the primitive acini (green arrow) and the islets cells with highly increased staining affinity for the apoptotic marker caspase 3 (black arrow) and (c) S2 group shows the primitive acini (green arrow) and the islets cells with densely stained caspase
Scale bars 20 µm

transmission in the descending reticular formation and spinal cord; but the strict technique of act of Soma is obscure in humans[30].

Carisoprodol was secreted in breast milk ranging from 2-4 times than it was in the mother's plasma[31]. Also, carisoprodol cause to an increased danger of birth defect through the first trimester of gestation[32]. In current study, pregnant rats which were preserved with low and high doses of carisoprodol caused many changes in pancreatic tissue of fetuses of S1 and S2 groups associated to group of control.

Many generative variations were observed in the pancreatic tissue of S1 group, pancreatic structures formed mostly from the exocrine acinar and ductal structures with ill-distinct endocrine counterpart, ill-distinct cell boundaries with

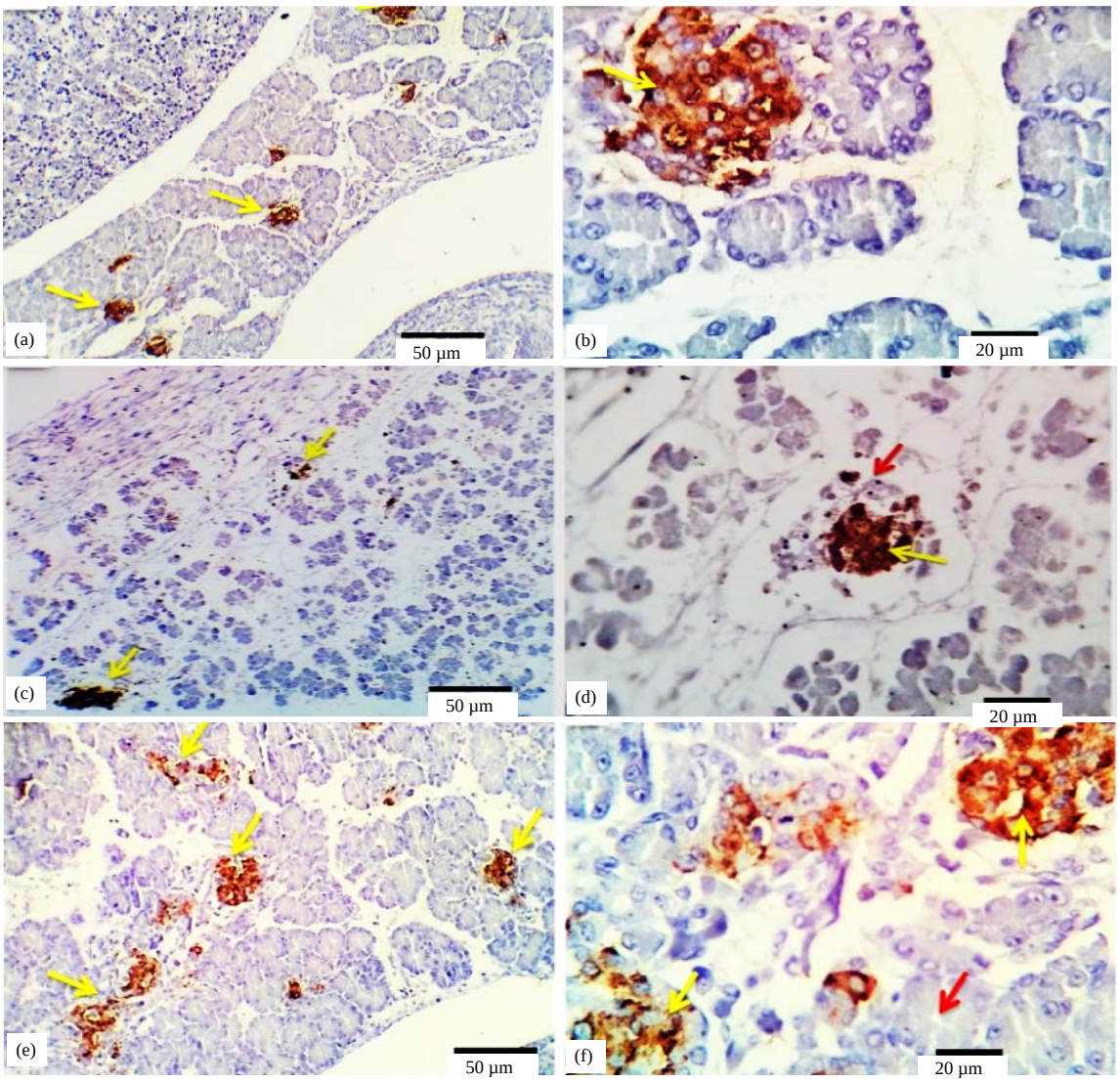

Fig. 8(a-f): Photomicrographs of fetal pancreas sections stained with insulin-marker. (a-b) Control group shows moderate number of randomly distributed islets of Langerhans with about 80-90% of β cells appear positively stained with the developer chromogen conjugate as cytoplasmic fine brownish granular materials (yellow arrows), (c-d) S1 group shows a few number of isles of Langerhans of a relative small to moderate sizes with about 60-70% of β cells appear staining reaction to the insulin-marker (yellow arrows), unstained cells appear oftenly at a peripheral location (red arrow) and (e-f) S2 group shows a moderate to large numbers and sizes of randomly distributed islets of Langerhans, a positive staining reactivity of β cells to the insulin-marker, about 80-90% of β cells appear positively stained as cytoplasmic fine brownish granular material (yellow arrows) and unstained cells (red arrow)
Scale bars 50 and 20 µm

nearly complete disappearance of the nuclei and or presence of some pyknotic ones, highly overfilled blood vessels which contained hemolysed blood cells and highly widened stroma in between the pancreatic acini. Carisoprodol showed well organized, healthy pancreatic structures with presence of moderate number of islets of Langerhans randomly distributed among well-formed pancreatic acini. But, highly dilated and congested blood vessel was detected; it contained numerous hemolysed RBCs and was surrounded by large hemorrhagic area and contained hemolysed blood cells and numerous hemosiderin granules. The results of the present work agree with results of Abouel-Magd[17] who recorded great hemorrhagic parts, highly extended and crowded blood vessels, many megakaryocytes and many deteriorated

changes in the fetal and maternal liver tissue post treatment with carisoprodol. Also, Abdelhafez and Abd Rabou[19] showed that usage of gravid rats with Soma led to much degenerative variations in the kidney cortex tissue of mothers and their fetuses.

In the present study, increased collagen fibers were detected in the cytoplasm of fetal pancreatic cells, pancreatic duct and islets of Langerhans of set S1. While, the pancreatic tissue of set S2 reported very amplified collagen bundles in and about the exocrine part, stroma in between them and islets of Langerhans post usage of pregnant mothers with carisoprodol and these outcomes agree with outcomes of Abouel-Magd[17] who noticed an increase in the collagen fibers in the fetal liver maternally treated with Soma. Also, Abd Rabou[16] found increased collagen fibers in the fetal esophageal tissue after treatment of their mothers with carisoprodol. Meyer *et al.*[33] reported that the toxic materials led to increased collagen fibers may be by cause of increase the defense feedback.

The result in present study presented enlarged PAS+ve materials in pancreatic cell of S1 and S2 groups. These results agree with those of Emam[18] who reported amplified PAS+ve materials in the lung of pregnant rats after treatment with carisoprodol. The increasing in polysaccharides in the current study was due to rise in thickness of these constituents or may be because of the rise in the RBCs after injuriousness.

The result of the present study showed increased staining affinity of total protein content in the cytoplasm of fetal pancreatic cells in S1 and S2 groups compared with group of control. Amplified staining affinity of the protein content in the current study come in concurrence with a previous study[34] which showed that treatment of pregnant rats with carisoprodol caused increase staining affinity of total protein content in the lung tissue of the pregnant rats and their fetuses.

Proteins are essentially involved in the building of the cell[35]. It is glucose control, insulin regulation and metabolism regulation[36]. The generation of ROS and consequent oxidative stress may be led to increased staining affinity of total protein[37].

The present work reported a highly amplified stain affinity of the amyloid- β protein in cytoplasm of fetal pancreatic cells and the blood vessels of group S1. Also, deeply stained amyloid precipitates were noticed in most pancreatic and vascular cells in the fetal pancreatic tissues of S2 set related to group of control. Abouel-Magd[17] found an increase in the amyloid protein in the liver cells, blood cells and necrotic regions after treatment of pregnant rats with carisoprodol. Also, fetal esophagus tissue of pregnant rats treated with carisoprodol reported amplified staining affinity of amyloid protein in the cells of esophagus[16]. Amyloid deposition caused dysfunction in mitochondria and creation of reactive oxygen species which caused apoptosis[39].

In fetuses maternally treated with the low dose of carisoprodol (S1 group) nuclei appeared markedly shrunk with decreased DNA materials in the pancreatic acini and pancreatic islet of Langerhans, while in those maternally treated with the high dose (S2 group), the nuclei became swollen with intensely stained DNA materials in the pancreatic acini and pancreatic islet of Langerhans related to the group of control. The results of the present research were supported by results of Abouel-Magd[17] who achieved numerous degenerative variations in the nuclei of liver tissue of fetuses and their mothers.

The reduction in the DNA materials in this study may be due to disruption of lysosomal membranes or due to stopped metabolism or to use them to promote new cells. This opinion is in agreement with those of Sakr and Shalaby[38].

Results of the present study demonstrated highly increased staining affinity for the apoptotic marker caspase 3 in the pancreatic cells of fetuses in S1 and S2 groups related to group of control. Caspase-3 is the major effector caspase for the detection of apoptotic pathways[39]. So, in this result, activation of caspase-3 was a marker for cell damage in the pancreatic fetal tissue. Apoptosis is an important process complicated in the damage of β cells[40].

The present work, recorded that most of the beta cells were positively stained for the insulin marker (80-90%) and insulin positive cells became more fewer with about 60-70% staining reaction to the insulin bio-marker at low dose of carisoprodol (Group S1) while, pointed out moderate to large numbers and sizes of distributed islets of Langerhans with a positive staining for the insulin marker (80-90%) in group S2. The results of this study agreed with results of El-Shaer and Abd El-Azez[41] who showed fewer positively stained beta cells for the insulin marker at toxic dose administrated group. No previous studies discussed the hazard of carisoprodol on cell of pancreas in the fetal rats.

CONCLUSION

Administration of carisoprodol (somadril) drug during pregnancy caused several histological, histochemical and immunohistochemical alternations in the fetal pancreatic tissues due to cross the drug from placenta to fetuses. Many alternations were found in the fetal pancreatic tissue such as pancreatic structures created especially from exocrine acinar and ductal structures with ill-distinguished endocrine

counterpart, ill-distinguished cell boundaries with nearly full passing of the nuclei and or existence of some pyknotic ones, much overfilled blood vessels which contained hemolysed blood cells and highly widened stroma in between the pancreatic acini.

SIGNIFICANCE STATEMENT

The results from this present study indicated that the administration of Carisoprodol during pregnancy caused histopathological, histochemical and immunological alternations in the fetal pancreatic tissue. This study will help the researcher to uncover the hazards of Carisoprodol on the fetal tissues during pregnancy.

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