

Histological and Histochemical Alterations Induced in Rats Fetal Esophageal Tissue Intoxicated Maternally with Carisoprodol

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ABSTRACT

Background: skeletal muscle relaxants are a varied set of drugs which treat pain of muscles or shudders from marginal musculoskeletal circumstances. **Aim of the work:** this work targeted to detect the histopathological and histochemical alternations in the rats fetal esophageal treated maternally with Carisoprodol (Soma). **Material and methods:** thirty gravid rats were branded into three sets: 1-Rats of the 1st group served as the control group and they were administered oral doses of distilled water, 2- Group of 10 pregnant rats served as the 2nd group and they were treated daily with 10.8 mg/100g/day b.wt. of Carisoprodol, 3-In group 3 pregnant rats were treated with 21.6 mg/100g body weight/day of Carisoprodol (treatment started from the 6th till the 20th day of gestation). Pregnant mothers were sacrificed and small sections of fetal rat esophagus were taken for the histopathological and histochemical purposes. **Results:** numerous histopathological and histochemical changes were detected in the fetal esophageal tissue of the two treated groups compared to the control group and the alternations were amplified with raising the doses of carisoprodol. **Conclusion:** treatment of the pregnant rats with carisoprodol caused dystrophic variations in the fetal esophageal tissue, so the usage of this medicine during gestation should be under strict protections.

Keywords: Carisoprodol, fetuses, pregnant rats, esophagus, histopathology and histochemistry.

INTRODUCTION

Skeletal muscle relaxants contain a diverse mixture of proxies with different buildings and instrument of achievement. These proxies are categorized as antispasticity or antispasmodic proxies; one of them is carisoprodol drug ^[1]. Carisoprodol is considered well than palliative and the dose which correspondence in efficiency actions is 350 mg and 250 mg. About less than one percent of patients who were treated with 250 mg dose of Soma informed sleepiness and no patient finished the medicine due to sleepiness ^[2]. It is essentially acts as skeletal muscle relaxant and it is thought to slab interneuron action by stimulating GABA-A receptors in descendent reticular creation and spinal rope ^[3]. This medicine has been increasingly harmed because of its meprobamate, exact substance that products tranquility through GABAA receptors ^[3].

In Norway, Carisoprodol was detached from shops in one May 2008, but it might still use by certain patients. It was branded from organized matter in numerous US states, then it energetic on 11 January 2012 ^[4]. It is extensively recycled in main care locations. The drug is found in markets by a diversity names (Soma, Sodal, Somalgit, Vanadam, Sonoma, Scutamil, Carisoma, Somacid, Mio Relax, Relacton-C, Relaxibys, Rela and Soridol) ^[4]. Soma is a neutral crystalline powder with white color and it is a chemical derivative of meprobamate with an aliphatic dicarbamate. It is soluble in many organic diluents, insoluble in vegetable oils, but soluble in water ^[5]. In the United States, carisoprodol is considered as the second greatest commonly set of muscle relaxant in 2000 ^[6]. Carisoprodol is used to increase or change belongings of other drugs (e.g. to rise pain killing

belongings of alcohol, avoid unease during cocaine feeding and assistance quiet persons) ^[7].

The cruelty of carisoprodol has amplified intensely in previous years. In certain patients who shortly stop carisoprodol consumption, a withdrawal disease happens. It appeared as seen with meprobamate withdrawal, signifying that they outcome from withdrawal from meprobamate collected with consumption of extreme carisoprodol and it is accomplished of moderating GABAA purpose, which may give to its abuse potential ^[4]. In addition, Carisoprodol may also harmfully affect cardiovascular functios (postural hypotension tachycardia and facial flushing) and gastrointestinal tract (vomiting, nausea, hiccup and epigastric distress) ^[8].

Esophagus is the first part of the alimentary canal that connects the laryngeal portion of the pharynx and the stomach. It lies ventral to the ventral cervical muscles and dorsal to the trachea. It is a highly specialized organ designed for transporting the food from the mouth to the stomach. Because of its position in the gastrointestinal tract it may be exposed to a variety of noxious stimuli ^[9].

Skeletal muscle relaxants characterize a varied pharmacotherapeutic group of drugs across several chemical modules that are physically dissimilar and these effective for spasticity and skeletal muscle contractions. This work aimed to determine the toxicity belongings of the oral treatment of Somadril (Carisoprodol) on the fetal rat esophageal tissue of albino rats.

MATERIAL and METHODS

Experimental animals:

Albino rats, male and female were used in the present work (150- 200 g). Before experiment animals were put in cages alone for two weeks under standard laboratory environment of light and temperature from June to August 2017.

The present study was approved from ethics committee of Al-Azhar University, College of Science, Cairo.

Gestation Course:

Estrous phase was performed according to Taylor ^[10]. Daily, vaginal marks were serene to examine the estrous phase. Two female rats were put in a cage with a single male overnight. Presence of sperms and vaginal plug means that breeding was established and that was reflected zero day of pregnancy ^[11].

Group of animals

Thirty gravid rats were classified into three groups (ten pregnant female rats in every set), animals of the control group(C) were taken oral doses of distilled water, S1 and S2 groups (pregnant rats treated with oral doses of carisoprodol equal to 10.8 mg / 100g, 21.6 mg/100g respectively of body weight every day for fifteen days from the 6th to the 20th day of pregnancy). Equivalent dose was adjusted according to Paget and Barnes ^[12]. All pregnant rats were dissected on the 20th day of gestation.

Drug

Soma drug was acquired as tablets of a combination of some components which contain carisoprodol 200 mg, caffeine 32mg and paracetamol 160 mg. The drug was acquired from Mina Pharm for Pharmaceuticals and Chemical Trades, Cairo, A.R.E.

Histopathological studies

After scarification, small pieces of esophagus were picked out for the histological and histochemical purposes. 5 µm sections were prepared and stained with hematoxylin and eosin ^[13], Mallory's trichrome technique for detecting collagen fibres ^[14], periodic acid Schiff's reagent for determine polysaccharides ^[15], mercuric bromophenol blue stain for finding of total protein ^[16], Feulgen reaction for detection of DNA ^[17] and Congo red stain for discovery the amyloid protein ^[18].

RESULTS

Normal layers of the esophageal tissue of fetuses in the normal group were observed in fig.1a. Numerous degenerated changes such as highly thickened mucosal layer were noted in S1 group (Fig.1b).

Highly thickened mucosal layer was noted in S2 group with numerous vacuolated cells (Fig.1c). Thin collagen fibres were supporting the different layers of the fetal esophageal tissue in C group (Fig.2a). Highly increased collagen fibres, hemorrhagic areas under the delaminated layers of the esophagus were detected in group S1 (Fig.2b). In addition, brightly red stained hemorrhagic areas were realized under the mucosal layer of fetal esophagus of S2 group (Fig.2c).

Esophagus of C group showed moderately stained PAS+ ve materials in all layers except the sub mucosa (Fig. 3a). Increased staining affinity of PAS +ve materials was demonstrated in all layers except sub mucosa of S1 group (Fig.3b), Also increased staining affinity was noticed in the internal border of mucosa layer in S2 group (Fig.3c).

Fig.4a showed deeply stained protein materials in all layers of the esophagus of C group except mucosa. Decreased staining affinity of total protein in was detected in the different layers of esophagus in S1 group (Fig.4b) and increased protein materials in the mucosal layer but, sub mucosa, discontinues muscle layers and serosa of S2 group were less stained (Fig.4c).

Fig. 5a showed moderately stained DNA materials in the different layers of esophagus of C group. Highly reduced DNA materials were detected in groups S1 and S2 (Figs.5b&c). Faintly stained amyloid-β protein were detected in the control group (Fig.6a). Increased staining affinity of amyloid protein in the different layers of esophagus of groups S1 and S2 (Figs.6b&c).

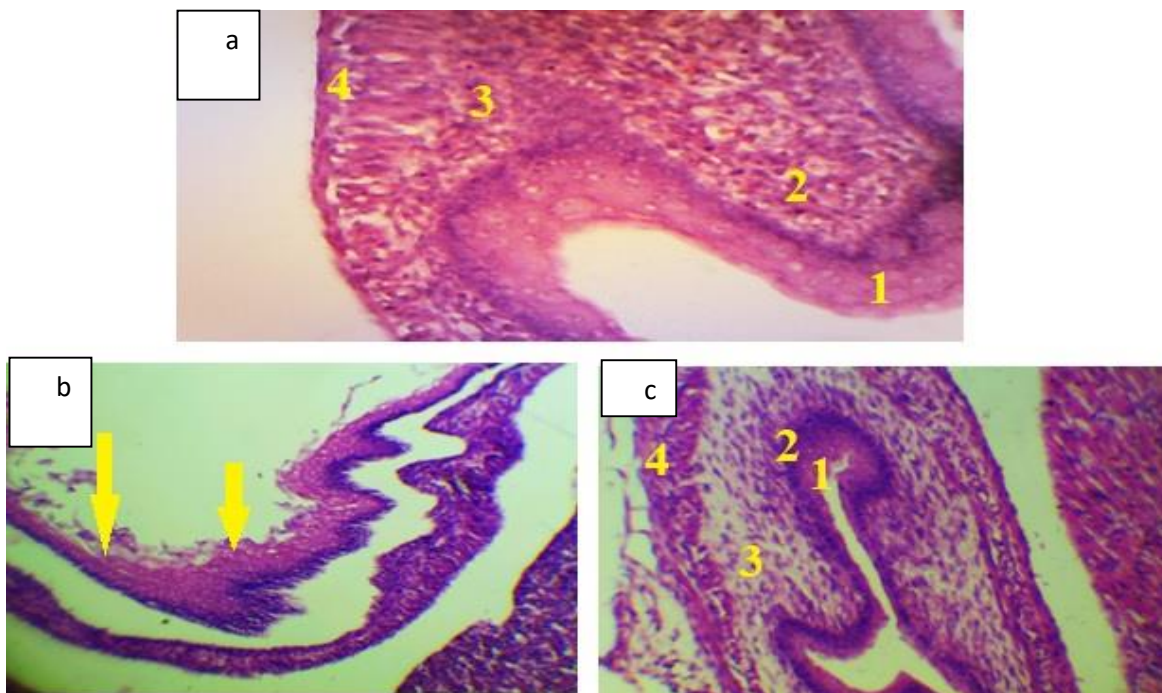


Figure 1 (a-c): photomicrographs of H and E stain of fetal esophagus (a) Group of control shows mucosa (1), sub mucosa (2), muscularis (3), serosa (4), (b) S1 group shows numerous degenerated changes such as highly thickened mucosal layer with lots of vacuolated cells (1), debris of degenerated mucosal cells can be detected in the lumen (2), delaminated mucosal and submucosal layers (3), and (c) S2 group shows highly thickened and vacuolated mucosal layer (1), highly distorted submucosal layer with hypo cellularity in it (2), discontinuous and ruptured muscle fibers in the muscularis layer (3) with ruptured serosa (4). (X=200)

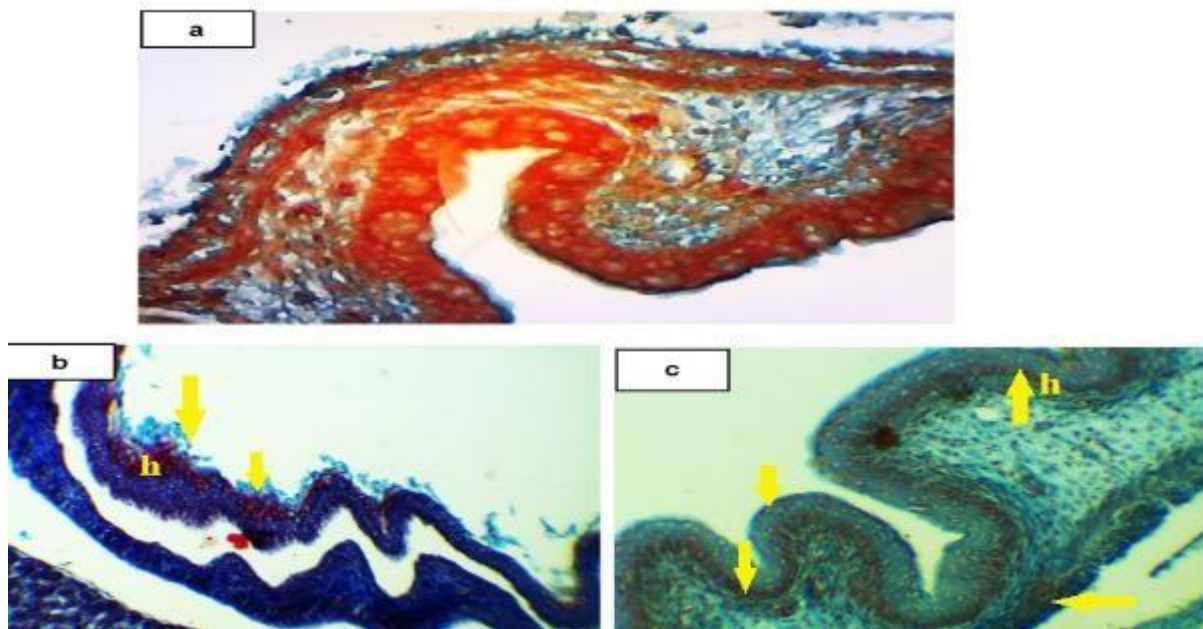


Figure 2(a-c): photomicrographs of Mallory's trichrome stain of fetal esophagus , (a) Group of control shows thin collagen fibres support the different layers of the esophagus, (b) S1 group shows highly amplified collagen fibres in all layers of the esophagus and in debris of degenerated mucosal layer (↑).Notice: hemorrhagic area under the delaminated layers of the esophagus (h), and (c) S2 group shows highly increased collagen fibres in the different layers of the esophagus (↑), with brightly red stained hemorrhagic area under the mucosa (h) . (X = 200)

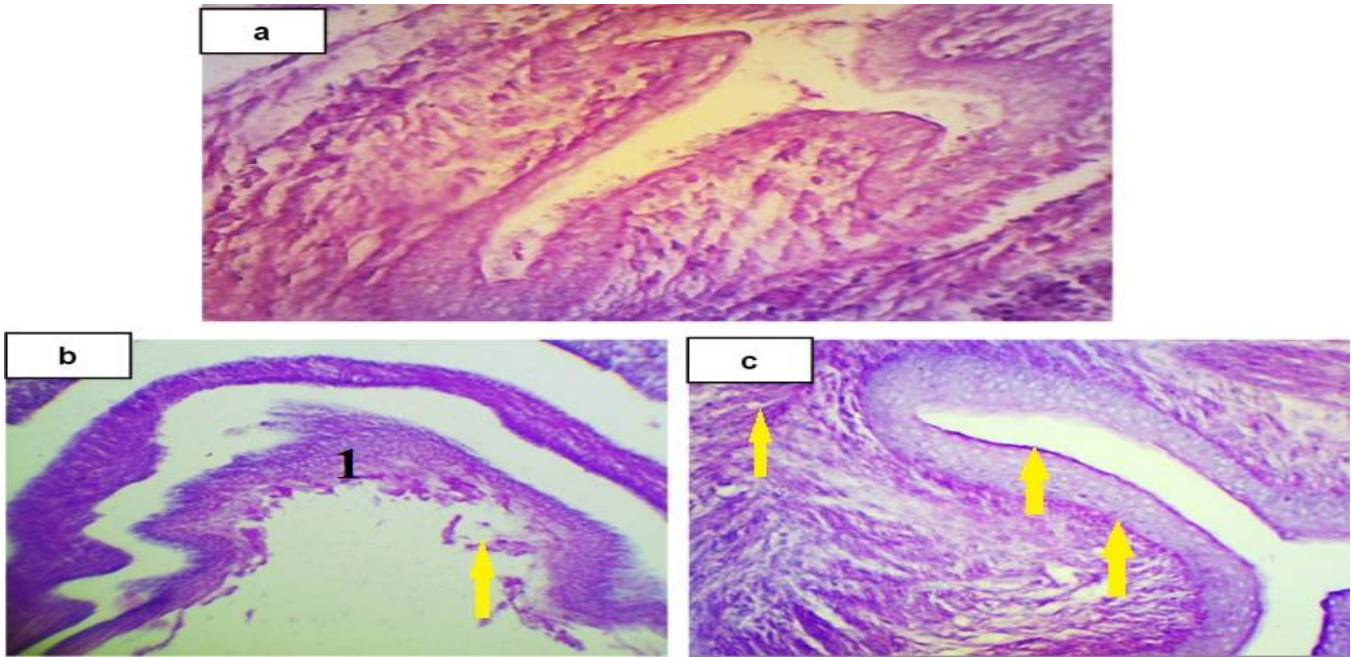


Figure 3 (a-c): photomicrographs of PAS stain of fetal esophagus (a) Group of control shows soberly PAS+ve supplies in the mucosa, muscularis and serosa layers with less stained submucosa, (b) S1 group shows increased of PAS +ve supplies in highly thickened mucosal layer and debris of it (↑) and in the muscularis layer with less staining affinity in submucosa (2) and (c) S2 group shows increased PAS +ve materials in the internal border of mucosa layer, expanded submucosa and some areas of muscularis (↑). (X=200)

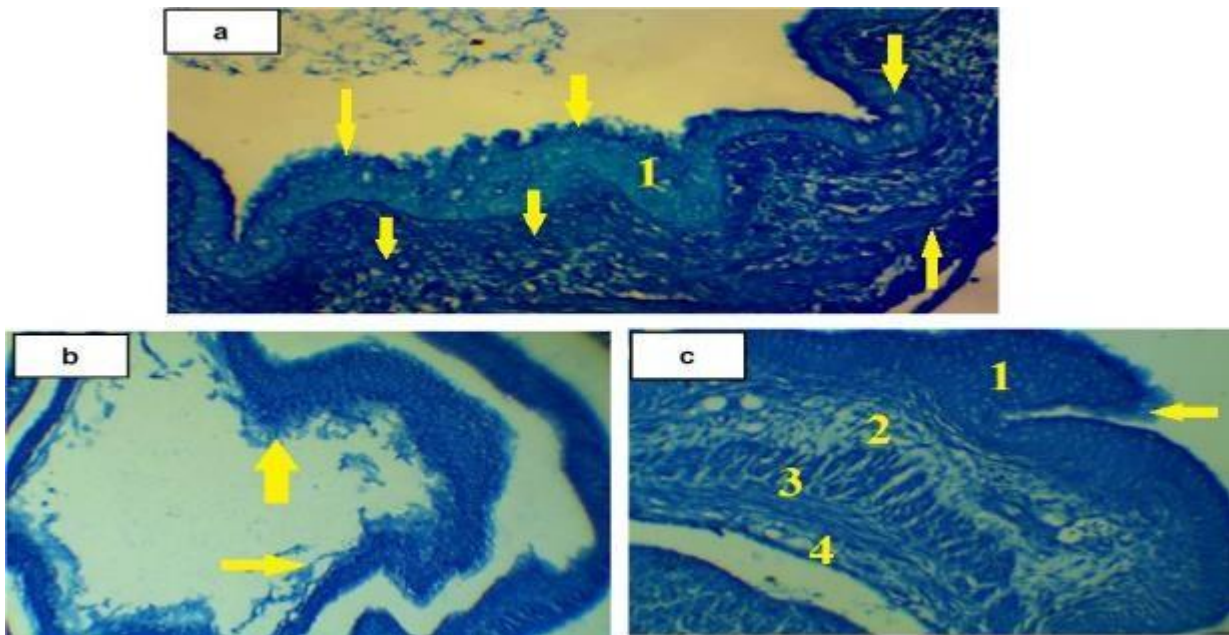


Figure 4 (a-c): photomicrographs of Mercuric bromophenole stained fetal esophagus sections of the (a) Control group shows deeply stained protein materials in the internal border of mucosa, submucosa, muscularis and serosa with less staining affinity in the cells of mucosa layer (↑), (b) S1 group shows reduced staining affinity of total protein in the different layers of esophagus. Notice: poorly proteinic materials in the debris of degenerated mucosal layer (1), and (c) S2 group showed increased protein materials in the highly thickened mucosal layer (1) with decreased staining affinity in the submucosa (2), discontinues muscle layers (3) and serosa (4).Notice: faintly stained debris of degenerated mucosa layer (↑). (X=200)

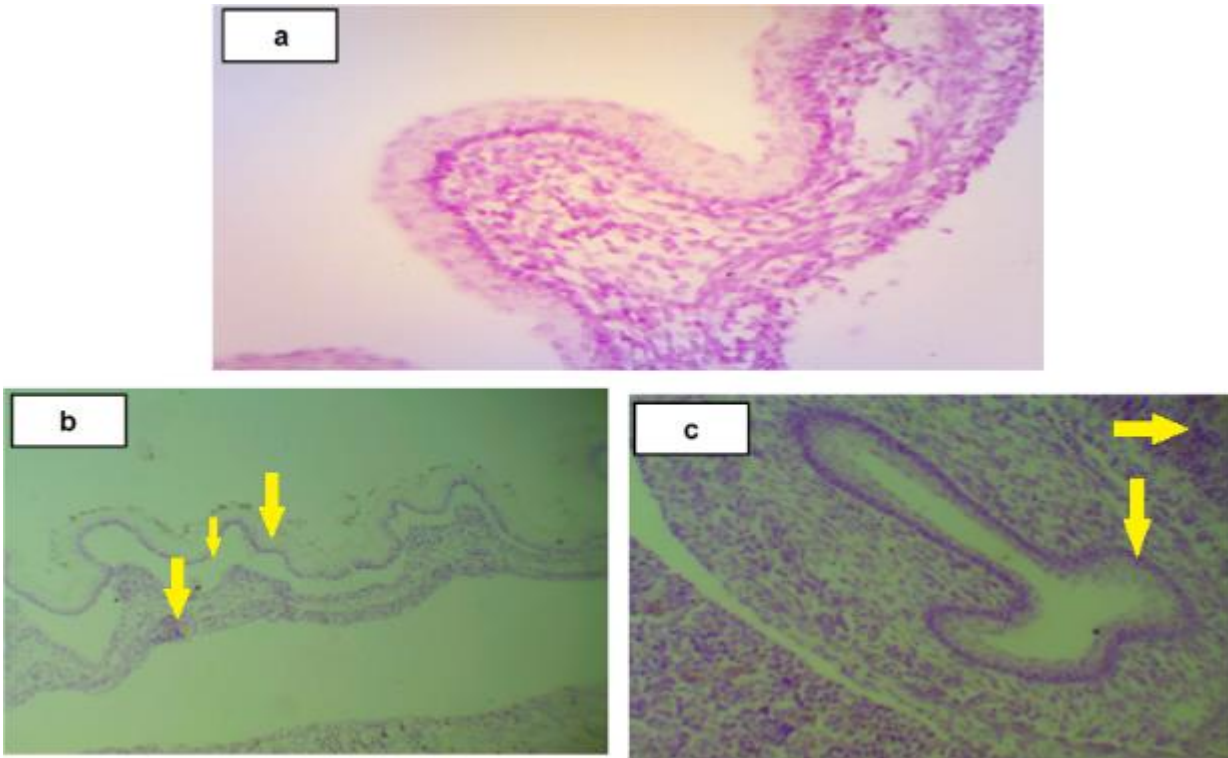


Figure 5 (a-c): photomicrographs of Fulgen DNA stained fetal esophagus sections of the (a) Control group shows moderately stained DNA materials in the different layers of esophagus, (b) S1 group shows highly reduced DNA materials (\uparrow) in the different layers of esophagus, and (c) S2 group shows increased staining affinity of DNA materials in different layers of the esophagus (\uparrow). (X=200)

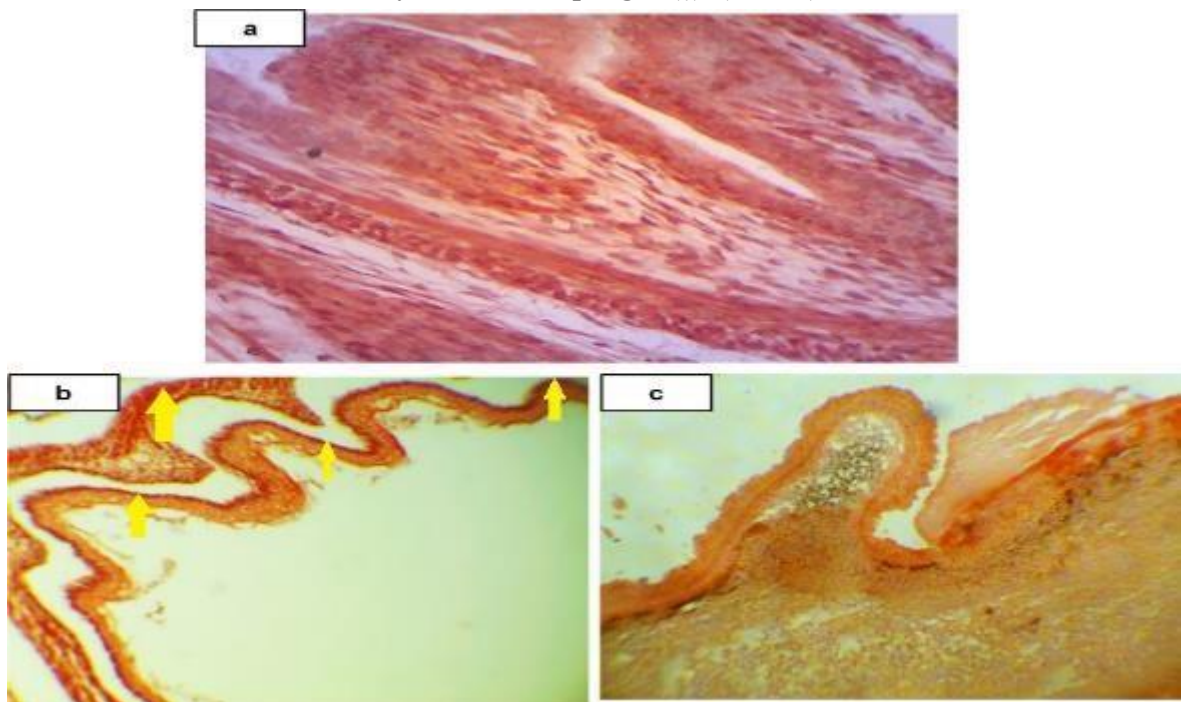


Figure 6 (a-c): photomicrographs of amyloid- β protein in fetal esophagus stained with Congo red of a) - Control group shows faintly stained amyloid- β protei, b) - S1 group shows increased staining affinity of amyloid protein in in the different layers of esophagus (\uparrow) and c) - S2 group shows increased staining affinity of amyloid deposits in the different layers of esophagus. (X=200)

DISCUSSION

In the present study, mothers which were treated with carisoprodol showed many degenerative deviations in the fetal esophagus of groups S1 and S2 compared to the control group.

Problems of tension in muscles and lower backbone pain can be resolved by carisoprodol ^[19]. The present study demonstrated highly thickened mucosal layer with lots of vacuolated cells, debris of degenerated mucosal cells in the lumen, delaminated mucosal and sub mucosal layers and highly distorted sub mucosal layer with hypo cellularity in it, discontinuous and ruptured muscle fibres in the muscularis layer with ruptured serosa after carisoprodol treatment. These investigations agree with those of **Anwar et al.** ^[20] and **Abouel-Magd** ^[21]. Treated rat with Somadril showed testicular vacuolation and degeneration and lessening in spermatozoa within the lumen and loss in normal architecture of the seminiferous tubules ^[20]. In instance report of dog, poisonous belongings of carisoprodol involved dejection in lung, minor sinus tachycardia, annexations and ataxia ^[22]. Also, Carisoprodol has been established to utilize opposing properties on mortal recital ^[23]. Congenital malformations during the first trimester of gestation may be occurred by carisoprodol ^[24]. These results agree with our results and results of **Abouel-Magd** ^[21] since fetal tissues maternally treated with carisoprodol were highly affected. These results don't agree with those of **Briggs** ^[25] who stated that carisoprodol did not cause developmental toxicity (structural anomalies, eurobehavioral deficits, growth restriction or death), but the long-term follow-up of carisoprodol appeared later in life. In the present work, increased collagen fibres were noticed in the fetal esophagus of S1 and S2 groups and in the degenerated mucosal layer with hemorrhagic areas under the delaminated layers of the esophagus of group S1. Brightly red stained hemorrhagic areas under the mucosa in the S2 group were noted. **Abouel-Magd** ^[21] found increased collagen fibres in liver tissue of fetuses of rats maternally treated with Carisoprodol during pregnancy. Collagen is essential for formation of ligaments, muscless, skin and blood vessels ^[26]. This may be due to encourages of expression of genes involved in collagen biosynthesis due to oxidative stress.

Results of this work showed increased PAS +ve materials in the fetal esophagus tissue in S1 and S2 groups, with less staining affinity in sub mucosa in the esophagus of S1 group. Decreased polysaccharides may be due to consuming high energy by increased stress on the organs.

Decreased stain of total protein in all esophageal layers with poorly proteinic materials were realized in the debris of degenerated mucosal layer in S1 group, but increased protein materials was noticed in the highly thickened mucosal layer with decreased staining affinity in the sub mucosa, discontinues muscle layers and serosa in S2 group. These results agree with those of **Abouel-Magd** ^[21]. The architecture of the cells contain mainly proteins ^[27]. It is associated with insulin regulation glucose control, regulation of metabolism and muscle building ^[28]. Decreased protein content in the different tissues was reported by **Al Gahtani** ^[29]. The reduction in protein could be qualified to lysosomal membranes disruption due to the effects of numerous toxicants and lead to freedom of hydrolytic enzymes in cytoplasm. Lysis and dissolution of goal material inside the cytoplasm due to occurrence of hydrolytic enzymes ^[30].

The present study presented decreased DNA materials in all layers of the fetal esophagus in groups S1 and S2. These results agree with those of **Abouel-Magd** ^[21]. The decrease in DNA in the present study might be owing to halted breakdown or to produce enzymes ^[31]. Dissolution of DNA in collapsed cell because of double dissimilar morphological forms of cells loss either by programmed cell death or necrosis ^[32].

Increased staining affinity of amyloid protein was noted in the present study in the different layers of esophagus of groups S1 and S2. Fetal liver tissue of mothers treated with carisoprodol showed extremely increased in amyloid protein in most liver cells, necrotic regions and blood cells ^[21]. Amyloid admission may lead to dysfunction in mitochondria and production of reactive oxygen species which lead to programmed cell death ^[33]. Other studies are needed to evaluate skeletal muscle relaxants medicines and their effect on the fetal growth through gravidity.

CONCLUSION

These results display that use of carisoprodol during pregnancy led to several histopathological alternations in the tissues of outcomes because of crossing of the drug from placenta to fetuses, so it caused opposing belongings on esophagus growth.

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