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Research Article
Toxicity of Somadril Compound on Fetal Ileum Tissues of Albino Rats

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Abstract

Background and Objective: Carisoprodol is a relaxant muscular-skeleton associated with sore muscles and appropriate studies have not been performed on carisoprodol effects on fetuses and mothers. This study has been conducted to clarify the treatment with a high and low dosage of carisoprodol (Somadril) on the histopathological, histochemical changes in the fetal ileum of the Albino rats. **Materials and Methods:** In the present research 30 adult pregnant rats have been used and divided into three classes (10 pregnant rats in each group), the first group was the group of Control (C). The 2nd and 3rd groups (S_1 and S_2) were treated with carisoprodol oral doses equating to 10.8 and 21.6 mg/100 g b.wt. per day, respectively. For 15 days from day 6-20 of pregnancy, groups S_1 and S_2 are administered. On the 20th day of pregnancy, the pregnant rats were sacrificed and small parts of fetal ileum for histopathological and histochemical studies. **Results:** Diverse histopathological and histochemical alternations were detected in the fetal ileum tissue of the two groups S_1 and S_2 after maternal treatment with high and low doses of carisoprodol compared to the control set. **Conclusion:** This study showed that several histopathological and histochemical deformities in the fetal ileum tissues were caused by the administration of carisoprodol.

Key words: Carisoprodol, fetuses, pancreas, histopathology, histochemistry, immunology, congenital malformations

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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

Somadril is considered a muscle relaxant, it uses as an auxiliary treatment in painful cases[1]. It is widely advised as adjunctive therapy in acute cases, but in recent years misuse of this drug has occurred[2]. And in America, it is frequently used[3]. Some studies found that is a possibility that it may lead to central nervous system depression and may lead to symptoms more difficult and annoying[4].

Somadril contains paracetamol, caffeine and carisoprodol[5]. There are numerous names in the Somadril market (Carisoma, Vanadam, Soma, Somacid, Somalgit, Sodol, Mio Relax, Sonoma, Scutamil and Soridol)[6]. Overdose, carisoprodol causes symptoms like shock, coma, breathing disorder and death[7]. It must be used within narrow limits depending on the health condition of patients, especially hepatic or renal functions whereas, it enters the liver and kidneys to complete the metabolism[8].

The ileum is known as the last portion of the small intestine in the higher vertebrates, such as mammals, creepers and aves[9]. In previous studies, Somadril caused many histological and histochemical changes in many tissues. In the hepatic tissue of mothers and fetuses, the somadril treatment of pregnant rats developed many significant pathological symptoms, including vascular ponds, multiple hemorrhage areas and cytological necrosis[10]. Certain drugs and some toxic chemicals may cause lung tissue damage, which is known as a toxicological problem. Using carisoprodol drug in pregnant rats led to many histological alternations in the kidney cortex tissue in both mother and fetal lung kidney cortex[11] and lung tissues[1].

According to previous studies, the use of carisoprodol in treating pregnant rats caused dystrophic changes in the fetal esophagus tissues from the placenta to fetuses due to the cross of the drug[12].

Concerning pregnant women, there are no studies that report this drug. It causes effects on fetal growth and postnatal survival which is found by some animal experiments. On the other hand, it is used to treat anxiety. Studies have also shown no link between maternal meprobamate use and increased risk of serious congenital malformations.

Also, limited literature was available on the clinical profile of the abuser of carisoprodol, this study aimed to find out the effect of carisoprodol on the histopathological and histochemical changes in the ileum tissue of fetal rats with a comparison of groups.

MATERIALS AND METHODS

Experimental animals: Total of 30 Animals in this experiment, adult albino rats, weighing 170-200 g on average, were used. The study was carried out at the Department of Zoology, Faculty of Science, Al-Azhar University, Egypt from July-August, 2017.

The males were housed in separate cages. Following that, 10 days were accommodated before the analysis began. During the experiment, they received water, *ad libitum* and rat chows. At night, males were put for mating with females. The presence of sperms was tested by vaginal smear every morning. When sperm was found, 0 days of gestation were considered.

Experimental design: In 3 sets (10 pregnant rats in each set) pregnant rats were rammed without a destiny. First (C): The purified water is handled orally by normal rats). The second series (S_1): Carisoprodol in distilled water was orally carried out at a dosage of 10.8 mg/100 g b.wt. in pregnant rat for fifteen days average from 6-20 days of pregnancy daily[10]. The third package (S_2): Carisoprodol was administered at a dose of 21.6 mg/100 g b.wt., in distilled water for fifteen days average from 6-20 days of pregnancy daily[10]. Similar to the technique of Paget and Barnes[13] of the human dosage, the dosage for the pregnant rats was planned. Within 24 hrs the latter drug department had abandoned them.

The Histopathological and Histochemical Examination: Both pregnant rats and fetuses were immolated and small ileum samples taken for histological and histochemical studies. They stained with Hematoxylin and eosin stain[14]. Collagen fibers were identified by Mallory trichrome stain[15], PAS was used for polysaccharide[16]. Complete proteins were also identified with mercuric bromophenol blue[15]. Feulgen was used to detect DNA[17]. The red Congo technique was used to detect amyloid protein[18].

RESULTS

Histopathological studies: Fetal ileum tissue of the control group revealed a normal histological structure (Fig. 1a). Fetal sections of the S_1 group showed elongated villi, some ruptured columnar cells, some degenerated in the muscularis and many nuclei in each layer deeply stained of ileum with internal bleeding in the lumen (Fig. 1b). Fetal sections of the S_2 group highly atrophied villi, degenerated areas in the muscularis, deeply stained nuclei in the multiple ileum layers (Fig. 1c). Examination of the Mallory trichrome stained in the fetal ileum in the control group showed thin collagen fiber

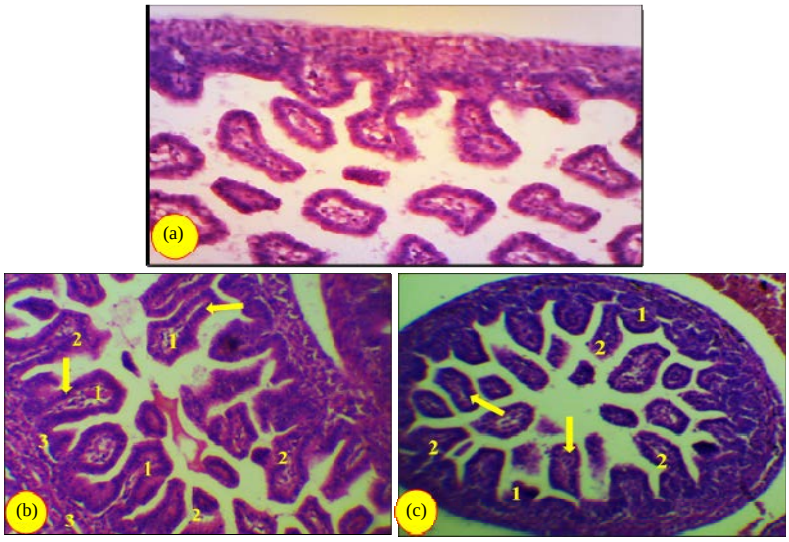

Fig. 1 (a-c): Image showing H and E stain of fetal ileum

(a) Control set indicates the normal histological structure of fetal ileum, (b) S_1 set indicates elongated villi of fetal ileum, (1) Some ruptured columnar cells, (2) Some degenerated areas are observed in the muscularis, (3) Lots of deeply stained nuclei are observed in the different layers (yellow arrow) with internal bleeding in the lumen of the ileum tissue (Black arrow) and (c) S_2 set indicates some villi are highly atrophied (1), Degenerated areas are observed in the muscularis, (2) Deeply stained nuclei were observed in the different layers (yellow arrow), X = 200

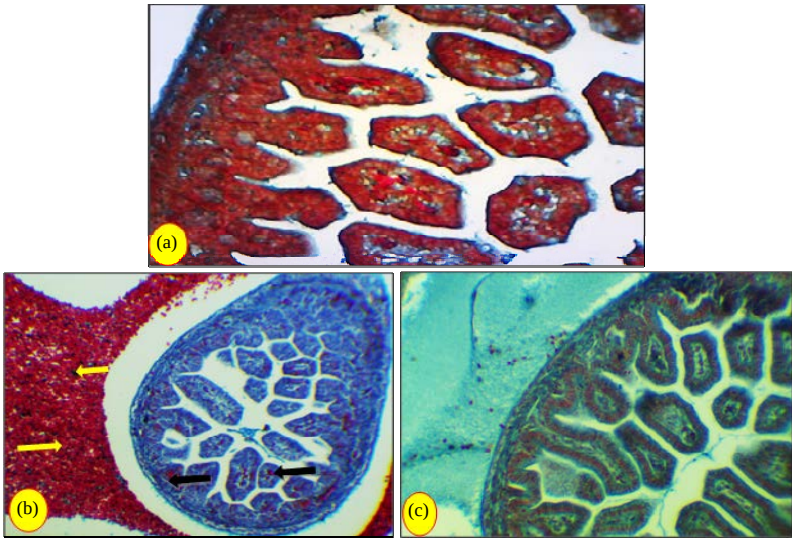

Fig. 2 (a-c): Image showing Mallory's trichrome stained sections of the ileum

(a) Control group shows thin bundles of collagen fibers supporting the different layers of the ileum tissue, (b) S_1 set indicates highly increased collagen fibers in the various ileum layers, the hemorrhagic area which contained brightly red stain blood cells (Black arrow) and a large hemorrhagic area around the ileum tissue which contained numerous hemosiderin granules (yellow arrow), (c) S_2 set indicates amplified collagen fibers mainly in the muscle layer, X = 200

bundles that sustain different layers of the ileum (Fig. 2a). The fetal ileum parts of the group S_1 were highly increased in collagen fibers with a large hemorrhagic area in the various layers of ileum and hemorrhagic region, which included some granules containing haemosiderin (Fig. 2b). In S_2, the collagen fibers in all the ileum layers were increased, especially in the muscle layer (Fig. 2c).

Histochemical Investigations: Fetal ileum of the control group showed well developed PAS +ve materials and in some villi, deeply stained cells (Fig. 3a). In all layers of ileum, the group S_1 showed an improvement in PAS +ve materials (Fig. 3b). In group S_2, an increase of PAS +ve materials with deeply stained goblets in the multiple layers of the fetal ileum were found in some villi (Fig. 3c). The Control group showed

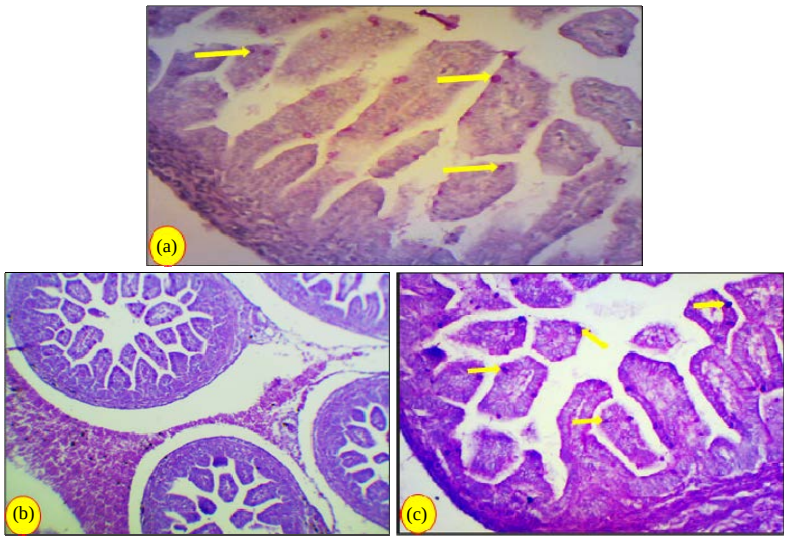

Fig. 3(a-c): Image showing PAS stain of fetal ileum

(a) C group shows well developed PAS +ve materials and in some villi deeply stained goblet cells (yellow arrow), (b) S_1 set shows an increase of PAS +ve materials in ileum layers, (c) S_2 set indicates amplified PAS +ve materials in the diverse fetal ileum with layer deeply stained goblet cells in some villi (yellow arrow), X = 200

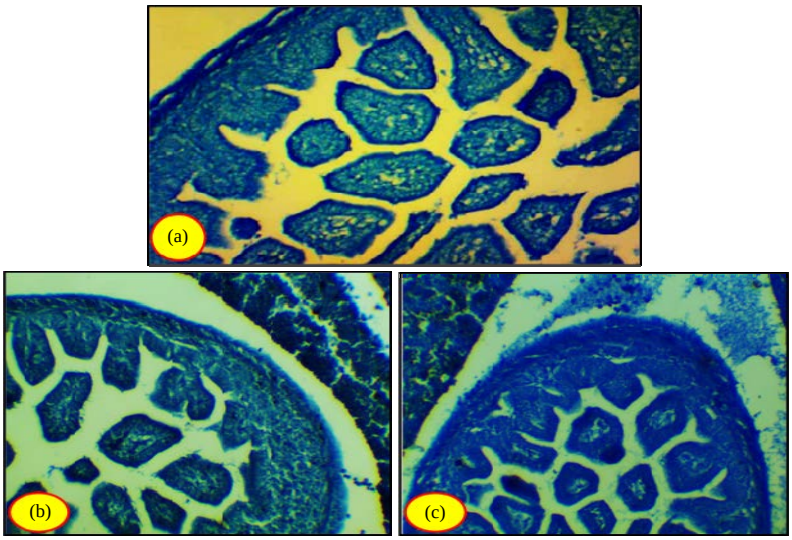

Fig. 4 (a-c): Image showing Mercuric bromophenol stained fetal ileum sections

(a) C group indicates moderately staining overall protein, (b) set S_1 shows decreased staining affinity over the entire protein in various fetal ileum layers with the deeply stained large hemorrhagic area around the ileum tissue and (c) set S_2 shows amplified protein materials in the various ileum layers with numerous hemorrhagic areas around ileum tissue, X = 200

moderately stained total protein content in the fetal ileum (Fig. 4a). In the S_1 group, the affinity of total protein in the various layers of the fetal Ileum has decreased with the deeply stained large hemorrhagic area around the ileum tissue (Fig. 4b). Increased protein materials in the different layers of fetal ileum with numerous hemorrhagic areas around the ileum tissue were detected in the S_2 group (Fig. 4c). Moderately stained DNA materials in nuclei of cells of the different layers of fetal ileum have been found in the control group (Fig. 5a). In fetal ileum of the S_1 group showed reduced DNA materials in nuclei of cells of the different layers of the ileum (Fig. 5b). Staining affinity of DNA materials in nuclei of cells of the different layers of the ileum of the S_2 group was increased (Fig. 5c). Faintly stained amyloid-β proteins were revealed in the fetal ileum tissue of control (Fig. 6a). In group S_1,

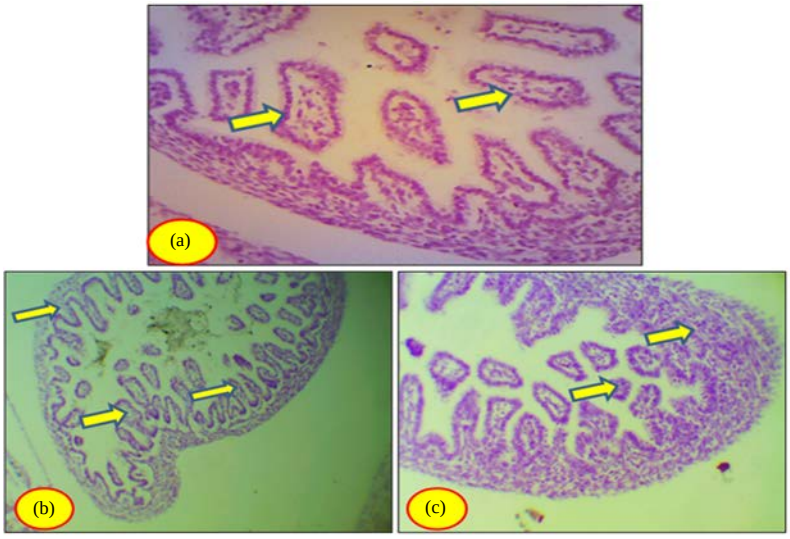

Fig. 5(a-c): Image showing Feulgen stained DNA materials in the sections of fetal ileum

(a) C group shows DNA materials moderately stained in the nuclei of cells of various ileum layers and other nuclei of the villi were deeply stained (yellow arrow), (b) S_1 group displays lower DNA material (yellow arrow) in nuclei of cells of the diverse ileum layers, (c) S_2 set indicates amplified in staining affinity of DNA materials in the nuclei of cells of the diverse layers of the ileum (yellow arrow), X = 200

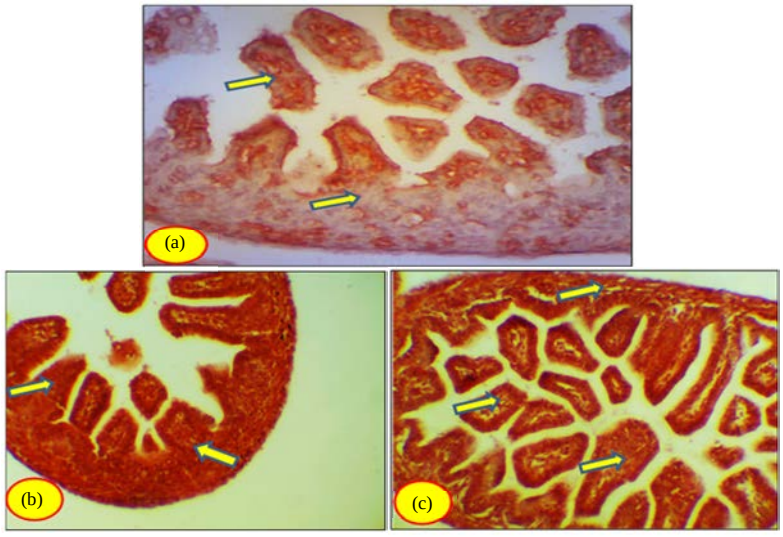

Fig. 6(a-c): Image showing amyloid-β protein in the fetal ileum dyed by Congo red stain

(a) Control set shows slightly dyed Amyloid-β protein, (b) Group S_1 indicates amplified amyloid protein staining affinity in different layers of the ileum (yellow arrow) and (c) S_2 shows increasing amyloids residue affinity in various ileum layers (yellow arrow), X = 200

improved amyloid protein staining preference in various ileum layers (Fig. 6b). Increased staining propensity of amyloid deposits is observed in the various layers of S_2 set ileum tissue (Fig. 6c).

DISCUSSION

The ileum has a high immune tissue quality. Peyer's patches lie in the mucosa that is an integral part of intestinal lymphoid tissue is a representative function. One Peyer is approximately 300 lymph follicles and is about 2-5 cm long. These bacteria are strong in the distal ileum and help to inhibit blood flow. This completes the chemical digestive process and consumes carbohydrates, vitamins and water[19].

Carisoprodol cultures muscle relaxation in the descending reticular fibers and spinal cord by obstructive interneuronal action[20]. Only or in groups with CNS depressants have overdosed of carisoprodol reported in fetuses[21].

Treatment of pregnant rats with carisoprodol in this study caused various degrading changes in the S_1 and S_2 fetal ileum tissue groups. These modifications involved: Elongated villi, highly atrophied villi, some ruptured columnar cells, some degenerated in the muscularis and many nuclei heavily stained in the different layers of ileum with internal bleeding in the lumen. In line with several previous studies, several degenerative liver deviations were triggered by the treatment of pregnant mothers with carisoprodol[10], kidney cortex[11], esophagus[12] and the lung tissues of fetuses[1]. Besides, Somadril treated rats showed vacuolation of testing and sperm deterioration in the lumen and normal seminiferous tubes injury[22]. The oxidative strain and the subsequent development of reactant oxygen types, which has been presumed to be the most significant toxic mechanism, may cause these flat histopathological deviations with carisoprodol treatments[23]. Reactive oxygen species and oxidative stress encourage the damage of cells by DNA fragmentation then apoptosis[24].

The present study revealed highly amplified collagen fibers of various layers in the fetal ileum of S_1 and S_2 sets. This increase in the collagen fibers agrees with the results previously reported by researchers[25,1,10-12]. The increase in collagen fibers results in a fast therapeutic cycle, whereby the collagens subtype accumulated secretion in the wound site replaces necrotic tissue in the proliferative phase of wound therapy[26]. The collagen is important for the creation of the ligaments, skin, muscles and blood vessels[27]. This may be triggered by the inspirations of genes in the biosynthesis of collagen due to oxidative stress.

The present research shows that the PAS +ve materials in the S_1 and S_2 fetal ileum tissue have increased and in group S_2 deeply stained goblet cells were detected in some villi. This increase in this research agreed with the results of Abd Rabou[12], but disagreed with the results of other researchers[10,11,1]. The accumulative of PAS +ve materials because of an increase in the red blood cells after the toxicity of somadril as stated by Suman *et al.*[28] and Abd El-Hady and AlJalaud[25].

The present study shows that the total protein tissue of S_1 and S_2 pregnant rats has increased its stain affinity but showed a reduced staining affinity of total protein in their fetuses compared to the control group. The present research showed that in the various layers of fetal ileum of S_1 set reduced staining affinity of the total protein with a broad hemorrhagic region deeply stained around the ileum tissue but increased protein materials with several hemorrhage areas around the ileum tissue were identified in the S_2 group in the different layers of the fetal ileum. Amplified staining affinity of total protein due to the generation of ROS and oxidative stress was observed[29]. The decrease in the total protein content was maybe due to the damage of organelles in the cells or due to decreased ribosomes[28].

In the current study, fetal ileum tissue of the S_1 group showed reduced DNA materials in cell nuclei in the various ileum layers. Amplified staining ability of DNA substances in cell nuclei of the different layers of the S_2 group of the ileum was obtained. Diminished DNA was also developed in the fetal lung tissue after somadril treatment[1]. DNA reduction was done due to a paused breakdown or due to the development of enzymes[30]. Besides, reduced DNA materials are due to active oxygen synthesis causing oxygen pressure and increased free radicals impacting DNA chains and contributing to cancer[28].

In this study, amyloid protein has shown increased stain sensitivity in the different fetal ileum layers in S_1 and S_2 sets. These observations are in agreement with the results of the previous studies[10,12,1]. The abnormal accretion of amyloid in the tissues may performance an important function in numerous neurodegenerative syndromes and cause amyloidosis[31]. The amyloid accretion is related to dysfunction in mitochondria and may cause the generation of ROS which can encourage a signaling path leading to apoptosis[32]. These results should be taken into consideration before using Somadril during gestation periods and future studies on the placenta can give a well understanding of the effect of Somadril use during the gestation period to recover the public health of embryos. According to the results of the current study, it is important to study the harms of skeletal muscle relaxants on fetuses.

CONCLUSION

The findings demonstrated that motherly use of carisoprodol was linked to dystrophic changes in tissues of fetuses and that increase risk of fetal malformations and placenta crossing of carisoprodol from pregnant rats to their fetuses may have negative effects on the ileum development.

SIGNIFICANCE STATEMENT

This study discovers the harmful effect of carisoprodol treatment on the fetal ileum tissue that can be beneficial for the prevention use of skeletal muscle relaxants drugs during pregnancy. This study will help the researcher to uncover the critical areas of carisoprodol drug during pregnancy that many researchers were not able to explore. Thus a new theory on the injury of skeletal muscle relaxants drugs on fetal tissues may be arrived at.

REFERENCES

1. Emam, N.M.M., 2020. Maternal and fetal toxicity of carisoprodol drug (somadril compound) on pulmonary tissues of albino. J. Biol. Life Sci., 11: 72-97.
2. Ralph, L.M., W. Look, S. Wheeler and H. Sacks, 2008. Double-blind, placebo-controlled trial of carisoprodol 250-mg tablets in the treatment of acute lower-back spasm. Curr. Med. Res. Opin., 24: 551-558.
3. Luo, X., R. Pietrobon, L. Curtis and L. Hey, 2004. P103. Prescription of nonsteroidal antiinflammatory drugs and muscle relaxants for back pain in the United States. The Spine J., 4: S89-S90.
4. Vo, K.T., H. Horng, C.G. Smollin and N.L. Benowitz, 2017. Severe carisoprodol withdrawal after a 14-year addiction and acute overdose. The J. Emergency Med., 52: 680-683.
5. Bramness, J.G., I. Buajordet and S. Skurtveit, 2008. The role of pharmacoepidemiological studies in the market withdrawal of carisoprodol (Somadril®) in Europe. Norsk Epidemiol., 18: 167-172.
6. Reeves, R.R., R.S. Burke and S. Kose, 2012. Carisoprodol: Update on abuse potential and legal status. Southern Med. J., 105: 619-623.
7. McIntyre, I.M., J. Sherrard and J. Lucas, 2012. Postmortem carisoprodol and meprobamate concentrations in blood and liver: Lack of significant redistribution. J. Anal. Toxicol., 36: 177-181.
8. Nebhinani, N., M. Aggarwal, S.K. Mattoo and D. Basu, 2013. Carisoprodol: An underrecognized drug of abuse in north India. General Hospital Psychiatry, 35: 89-92.
9. Guillaume, J., S. Kaushik, P. Bergot and R. Metailler, 2001. Nutrition and Feeding of Fish and Crustaceans. Praxis Publishing Ltd., Chichester, West Sussex, UK, Pages: 408.
10. Abouel-Magd, M.H., 2018. Maternal and fetal toxicity of carisoprodol. Egypt. J. Hosp. Med., 71: 2322-2350.
11. Abdelhafez, H.M. and M.A. Abd Rabou, 2019. Carisoprodol (somadril) induced histological and histochemical changes in renal cortex of pregnant rats and their fetuses. Egypt. J. Histol., Vol. 42. 10.21608/EJH.2019.19917.1203.
12. Abd Rabou, M.A., 2019. Histological and histochemical alterations induced in rats fetal esophageal tissue intoxicated maternally with carisoprodol. Egypt. J. Hosp. Med., 75: 2254-2260.
13. Paget, G.E. and J.M. Barnes, 1964. Interspecies Dosage Conversion Scheme in Evaluation of Results and Quantitative Application in Different Species. In: Evaluation of Drug Activities: Pharmacometrics, Laurence, D.R. and A.L. Bacharach (Ed.)., Academic Press, New York, ISBN: 9781483263465, pp: 160–162.
14. Bancroft, J.D. and M. Gamble, 2008. Theory and Practice of Histology Techniques. 6th Edn., Churchill Livingstone, London, ISBN: 0443102791, pp: 83-92.
15. Pearse, A.G.E., 1972. Histochemistry: Theoretical and Applied. 3rd Edn., J.A. Churchil, London, Pages: 1518.
16. Drury, R. and E. Wallington, 1980. Carleton's Histological Technique. 5th Edn., Oxford University Press, Oxford, New York, Toronto, Pages: 195.
17. Kiernan, J.A., 2015. Histological and Histochemical Methods, Theory and Practice. 5th Edn., Pergamon Press, New York, ISBN 978 1 907904 32 5, Pages: 592.
18. Valle, S., 1986. Special stains in the microwave oven. J. Histotechnol., 9: 237-239.
19. Nigam, Y., J. Knight and N. Williams, 2019. Gastrointestinal tract 4: Anatomy and role of the jejunum and ileum. Nursing Times, 115: 41-44.
20. Robertson, M.D. and L.J. Marinetti, 2003. Carisoprodol-effects on human performance and behavior. Forensic Sci. Rev., 15: 3-9.
21. Witenko, C., R. Moorman-Li, C. Motycka, K. Duane, J. Hincapie-Castillo, P. Leonard and C. Valaer, 2014. Considerations for the appropriate use of skeletal muscle relaxants for the management of acute low back pain. Pharm. Ther., 39: 427-435.
22. Ali, K.A., L.S. Salih and S.M. Abdulkareem, 2017. Histo-physiological study of some parts of organs treated with somadril drug. J. Raparin Univ., 4: 33-44.
23. Rajapakse, N., M.M. Kim, E. Mendis and S.K. Kim, 2007. Inhibition of free radical-mediated oxidation of cellular biomolecules by carboxylated chitooligosaccharides. Bioorg. Med. Chem., 15: 997-1003.
24. Ostrovsky, S., G. Kazimirsky, A. Gedanken and C. Brodie, 2009. Selective cytotoxic effect of ZnO nanoparticles on glioma cells. Nano Res., 2: 882-890.
25. Abd El-Hady, A.M. and N.A. Al Jalaud, 2015. Therapeutic effects of olive leaf extract or bone marrow mesenchymal stem cells against lung damage induced in male albino rats exposed to gamma radiation. Egypt. J. Hosp. Med., 61: 685-699.
26. Suvik, A. and A.W.M. Effendy, 2012. The use of modified Masson's trichrome staining in collagen evaluation in wound healing study. Malaysian J. Vet. Res., 3: 39-47.
27. Lamidi, I.Y. and I.O. Akefe, 2017. Mitigate effects of antioxidants in lead toxicity. Clin. Pharmacol. Toxicol. J., 1: 1-9.
28. Suman, S., O.C. Rodriguez, T.A. Winters, A.J. Fornace, C. Albanese and K. Datta, 2013. Therapeutic and space radiation exposure of mouse brain causes impaired DNA repair response and premature senescence by chronic oxidant production. Aging, 5: 607-622.
29. Sakr, S.A. and S.Y. Shalaby, 2011. Ginger extract protects metalaxyl-induced histomorphological and histochemical alterations in testes of albino mice. J. Applied Pharm. Sci., 1: 36-42.

30. Abuo El Naga, N.A. and M.A. Abd Rabou, 2012. The possible protective role of bone marrow transplantation on irradiated mothers and their fetuses. Stem Cell, 3: 8-30.
31. Pulawski, W., U. Ghoshdastider, V. Andrisano and S. Filipek, 2012. Ubiquitous amyloids. Appl. Biochem. Biotechnol., 166: 1626-1643.
32. Kadowaki, H., H. Nishitoh, F. Urano, C. Sadamitsu and A. Matsuzawa *et al*., 2005. Amyloid β induces neuronal cell death through ROS-mediated ASK1 activation. Cell Death Differ., 12: 19-24.