Assiut Vet. Med. J. Vol. 41 No. 81, April 1999.

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EFFECT OF LEAD NITRATE ON THE MYENTERIC PLEXUS OF NILE CATFISH, *CLARIAS LAZERA*

(With 4 Figures)

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(Received at 23/3/1999)

تأثير نترات الرصاص على مجموعة العقد العصبية لأمعاء أسماك القراميط النيلية

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تم وصف مجموعة العقد العصبيه لأول مره فى الجزء الأمامى والأوسط والخلفى لأمعاء أسماك القراميط النيليه وكذلك تأثير نترات الرصاص على هذه العقد بإستخدام الميكروسكوب الضوئى. إستخدمت فى هذه الدراسه ٤٢ سمكه تم تقسيمها إلى ٤ مجاميع. تم حقن هذه المجاميع عن طريق الحقن المعدي بعد تخدير الأسماك. تم حقن المجموعه الأولى يومياً وعددها ١٢ بتركيز ٤ جزء فى المليون من نترات الرصاص المذاب فى المياه المقطره يومياً. أما المجموعه الثانيه (١٢ سمكه) فحقنت بتركيز ٨ جزء فى المليون من نترات الرصاص المذاب فى المياه المقطره يومياً وأخيراً المجموعه الثالثه فتعرضت إلى ١٦ جزء فى المليون من نترات الرصاص المذاب فى المياه المقطره يومياً. تم حقن ضوابط التجربه (٦ سمكه) بالمياه المقطره يومياً. تم أخذ الجزء الأمامى والأوسط والخلفى لأمعاء الأسماك لكل من المعاملات وضوابط التجربه وذلك بعد أسبوعين وأربع أسابيع من الحقن المعدى ثم تم ثبيت العينات فى محلول البوان وقطعت وصبغت بالهيماتوكسلين والايوسين وصبغة اللكسول الأزرق. أظهرت الدراسه وجود مجموعة العقد العصبيه بين منطقة عضلات الأمعاء فى الجزء الأمامى والأوسط والخلفى لأمعاء الأسماك. كان تأثير نترات الرصاص واضحاً عند إستخدام ١٦ جزء فى المليون وخاصة بعد أربع أسابيع من الحقن وذلك عند مقارنتها بضوابط التجربه. تمثلت التغيرات فى تنكرز أنوية الخلايا العصبيه وفقدان أجسام نيسل وكذلك الهدبيات العصبيه Denderitis. نتائج هذه الدراسه قد تشير إلى أن تأثير نترات الرصاص على العقد العصبيه قد يكون معتمداً على زيادة تركيز الرصاص.

SUMMARY

Presence of myenteric plexus in the intestine of catfish has been reported for the first time. Furthermore, the histopathology of these plexus after being exposed to lead has not yet been described. In this study, thirty-six fish were exposed to 4, 8, and 16 ppm of lead nitrate daily for four weeks by gastric gavage. Six control fish received deionized distilled water by the same route. The intestines of both exposed and non-exposed fish were taken at two and four weeks post-administeration with lead and processed for light microscopy. The study showed that the myenteric plexus were found in the anterior, middle, and posterior parts of catfish intestine. Administeration of 8 and 16 ppm of lead nitrate resulted in pyknosis and karyolysis of the neuronal nuclei, loss of denderites and loss of Nissel bodies.

Key words: Lead, Myenteric Plexus, Catfish.

INTRODUCTION

The mammalian myenteric plexus (Auerbach's) are clusters or aggregates of neurons, sattellate in shape, having one axon but more than one denderites, which tends to form ball-like structure. These clusters are located between the muscular layer of intestine. These plexus are terminal (intramural, visceral) and belong to the parasympathetic division of the autonomic nervous system. The major role of these plexus is the control of the gastrointestinal motility in both fasting and fed states (Telford and Bridgman, 1995).

In mammals, there are several reports on the alterations of myenteric plexus in response to different causes. For example, meconium ileus of newborn infants is beleived to be due to immaturity of the myenteric plexus in the ileum (Toyosaka et al., 1994). Moreover, the myenteric plexus was considered a possible site of origin for gastrointestinal tumors in human (Walsh and Bodsurtha, 1990). Morphometeric measurements have shown decrease in the number of neurons of human oesophagus in relation to age (Meciano et al., 1995). In horse, equine myenteric

ganglionitis was described as neuronal degeneration, chromatolysis, cytoplasmic vacuolization, and decrease number of neurons (Burns et al., 1990). Chemical degeneration of intestinal nerves induced by cobalt chloride in dogs, also has been reported (Frantzides et al., 1990).

In fish, there are no reports available on the presence of myenteric plexus in their intestine. The purpose of this study was to identify the presence of myenteric plexus in the intestine of Nile catfish, (*Clarias lazera*) and to describe the histopathological changes of these plexus in catfish exposed to different concentrations of lead nitrate by gastric gavage administeration.

MATERIAL and METHODS

Fourty-two adult Nile catfish, (*Clarias lazera*) were collected from the River Nile, Assiut, Egypt. Their average body weight was 60 g and measured 40 cm in length. Fish were kept in aquaria containing 40 L dechlorinated municipal static water for two weeks. Water was exchanged daily during the experiment and acclimation period.

Methods:

Experimental design:

Thirty-six Nile catfish, *Clarias lazera* were randomly assigned into three groups in a separate glass aquaria. Each aquarium had twelve fish, which represented the treated groups. Six catfish were kept under the same conditions and represented the control group. Aquaria were provided with continuous areation during acclimation and experimental periods. Fish were fastened just the first day prior to the initial exposure. Water quality parameters were monitored weekly. The water temperature was 25 ± 1 during acclimation and experimental periods. The lead content in the water used in the laboratory was estimated previously using atomic absorption spectrophotometer (Zaki et al., 1994).

Fish were anaesthetized using MS_{222} obtained from (Sigma Chemical Co., St Louis, Mo, USA) before gavage. The first, second, and third groups received 4, 8 and 16 ppm of lead nitrate dissolved in deionized distilled water, respectively. Each fish recived 1 ml of the above concentrations daily for four weeks. The

gastric gavage was carried out by the use of a plastic tube connected with 5 ml syringe. Control group received 1 ml deionized distilled water daily for four weeks by the same way described.

Sampling and Histopathology:

Fish of treated and control groups were killed by pithing the brain tissue. Samples were taken at the end of the second and fourth weeks post-administration. Representitive samples of the intestine of each group (n = 6 at two weeks; n = 6 at four weeks) were taken, divided into anterior, middle, and posterior, and fixed immediately in Bouin's fixtive. Samples were dehydrated, embedded in paraffin, sectioned at 4-6$_u$, stained with H&E, and Modified Luxol Fast Blue (Bancroft et al., 1994), and examined by light microscopy.

RESULTS

The lead content in the municipal water used in this study was 0.04 ± 0.012 mg/l.

Clinical signs and behavior changes:

There were no obvious clinical signs observed during the four weeks exposure to lead nitrate.

Histology of the myenteric plexus in catfish intestine:

The myenteric plexus in this study was found in the anterior, middle, and posterior parts of the intestine examined. The neuron in this plexus had nerve cell body with distinct large nucleus and bluish stained Nissel bodies. Denderites were coiled and stained blue in colour. These clusters were found dividing the muscular layer (Fig. 1 a,b).

Gross lesions of exposed fish to lead nitrate:

Fish exposed to 8 and 16 ppm of lead nitrate at four weeks post-administration showed haemorrhagic intestinal mucosa with yellowish material in their lumen. Fish exposed to 4 ppm did not show any gross lesions.

Histopathology of exposed fish to lead nitrate:

Catfish exposed to 4 ppm of lead nitrate showed no histo-pathological changes in the myenteric plexus examined in

comparison with the control group. Catfish received 8 and 16 ppm of lead nitrate had alterations, which were obvious in fish exposed to 16 ppm at four weeks post-administeration than those exposed to 8 ppm of lead nitrate. These alterations were not observed at two weeks. The neurons of the myenteric plexus had lost their contour and became irregular in shape (Fig. 2). Neuronal pyknosis and karyolysis occurred in catfish exposed to 16 ppm at four weeks post-administration (Fig. 3). Moreover, loss of Nissel bodies and loss of denderites were observed (Fig. 4).

DISCUSSION

In mammals, the physiologic importance of the myenteric plexus has been studied with relation to intestinal absorption, secretion, and motility (Frantzides et al., 1987). Moreover, the pathological alterations of the myenteric plexus have been reported in newborn infants, human, and horse, and resulted in different syndromes (Toyosaka et al., 1994; Walsh and Bodurtha, 1990; Meciano et al., 1995). These reports reflect the importance of these plexus to consider.

The present study showed the existence of the myenteric plexus in Nile catfish, *Clarias lazera* intestine, which is not reported previously.

Lead is known to produce ulcerative, necrotic, and haemorrhagic gastroenteritis (Haschek, 1991). Therefore, lead was the choice to study its effect on the myenteric plexus of catfish. Catfish exposed to lead nitrate showed alterations in the myenteric plexus in comparison with control group. In this study these changes appeared to be a dose-dependent. The karyolysis and loss of Nissel bodies and loss of dendrites observed in catfish exposed to 8 and 16 ppm of lead nitrate at four weeks post-administration may suggest a direct effect of lead had occur. In the present study, there were no clinical signs observed in the exposed fish. This shows that higher doses than 16 ppm of lead in Nile catfish, may required to induce obvious signs.

CONCLUSION

This study reported the occurrence of the myenteric plexus in the anterior, middle, and posterior parts of Nile catfish, (*Clarias lazera*) intestine. Lead nitrate seems to affect the myenteric plexus in a dose-dependent way.

FIGURES LEGENDS

Fig. 1 (a): Control intestine of Nile catfish showing the presence of myenteric plexus (My) between the muscular layer (m). H. & E. X 32.

(b): Higher magnification showing the nerve cell body with visible nucleus (P) and denderites (d) of the myenteric plexus. H. & E. X 128.

Fig. 2: Catfish intestine exposed to 16 ppm of lead nitrate for 4 weeks. The neurons of the myenteric plexus were irregular in shape and lost their denderites (arrows). H. & E. X 320.

Fig. 3: Catfish intestine exposed to 16 ppm of lead nitrate for 4 weeks. The neurons of the myenteric plexus showing shrinked deeply stained cell bodies and nuclear pyknosis (arrows). H. & E. X 320.

Fig. 4: Catfish intestine exposed to 16 ppm of lead nitrate for 4 weeks. The neurons of the myenteric plexus showing loss of Nissel bodies and margination of nuclei (arrows). H. & E. X 320.

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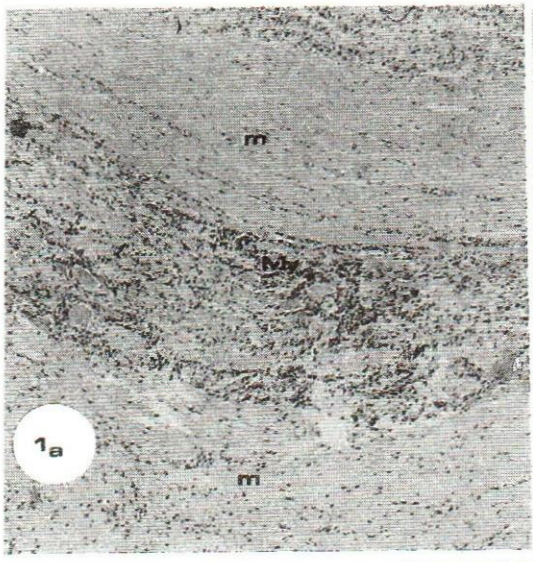
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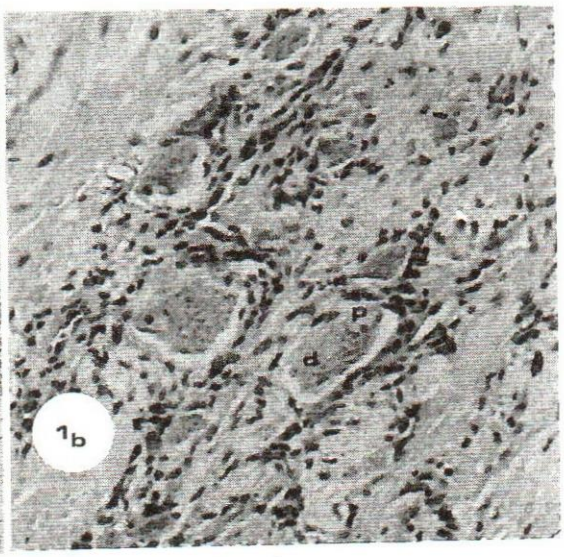
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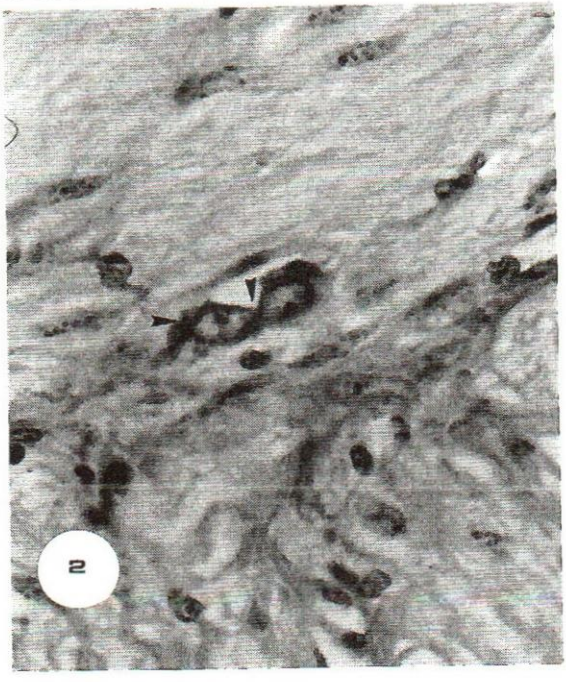
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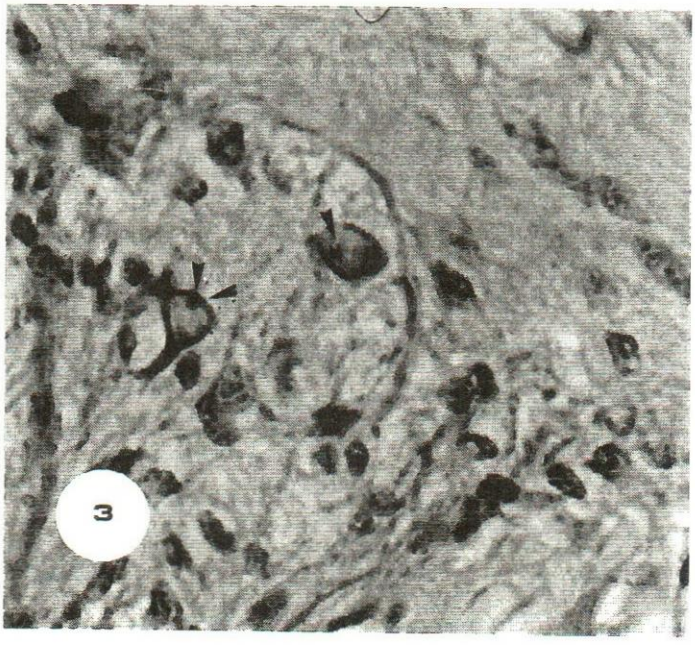
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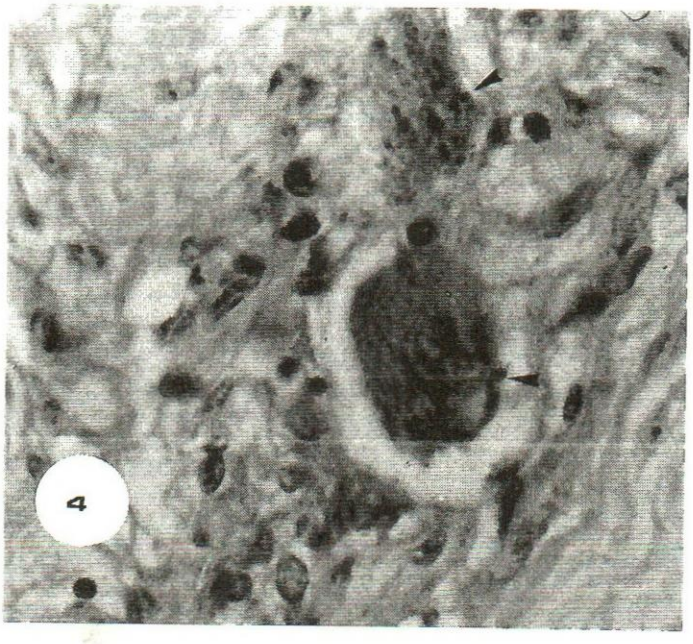
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