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IMPACT OF UV-A IRRADIATION ON THE INTESTINE OF FRESHWATER CRAYFISH *PROCAMBARUS CLARKII*.

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***Procambarus clarkii* is a freshwater crustacean which spread all over the River Nile. It has become an important crustacean model organism in research on animal behaviour, environmental stress, toxicity and its economic role. Ultraviolet radiation (UVR) has negative effects on aquatic organisms. The present study was designed to evaluate the effects of UV-A irradiation on the intestine of the crayfish *Procambarus clarkii*. To carry out the investigation, specimens were firstly adapted in the Lab, then classified into three groups. The first group was chosen as the control (untreated group) and the other two groups were the treated ones. The first treated group was irradiated for 15 minutes of UV-A for two weeks while the second treated group was irradiated for 30 minutes of UV-A for two weeks. Each treated group was lifted for two weeks for recovery after irradiation. After treatments, the intestine including circular and longitudinal muscles were examined. The structural integrity of the epithelium and myoepithelium of the intestine of the control and treated groups were assessed using histological, histochemical and electron microscopic techniques. The significance changes of histological and histochemical were observed in treated animal and discussed. The study revealed that after 15 min. of UV-A irradiation for two weeks, *P. clarkii* showed signs of damage of intestine and the damage increased with increasing the time of irradiation for 30 min. The damage included intestinal epithelial cells with cell membrane, irregular structure of cuticular layer and absence of organoids seen by electron microscopy. These necrotic effects of UV-A in intestinal cells in irradiated animals may cause lethal lesions. Incomplete recovery was observed in each group.**

1-Introduction

Crayfish *Procambarus clarkii* is one of the worldwide distributed invertebrates originated in Western Asia and Eastern Europe (Sepici-Dincel *et al*., 2013) [1]. Sometimes called Red swamp (Girard, 1852) [2], crayfish/crawfish and Louisiana crayfish/craw fish (Gherardi and Panov, 2006) [3]. They are fresh water crustaceans resembling small lobsters (Zaglol and Eltadawy, 2009) [4]. It was introduced worldwide and has become the dominant freshwater crayfish in almost all areas where it lives; it was introduced to River Nile, Egypt (Huner and Skurdal, 1996 [5]; El-Shaikh *et al*., 2005 [6] and Desouky *et al*., 2013 [7]) through a commercial aquaculture in Giza (Manial-Shiha), in the early 1980's. They greatly spread all over the River Nile in Egypt and its tributaries (Zaglol and Eltadawy, 2009 and El-Bakary and Sayed, 2011 [8]). *P. clarkii* is hardy warm water

crayfish that is typically found in marshes, rivers, slow flowing water, reservoirs, irrigation systems and rice fields (Saad and Habashy, 2002 [9]; Zaglol and Eltadawy, 2009 [4]; Sua´rez-Serrano *et al*., 2010 [10]; Casellato and Masiero, 2011 [11]; Chucholl, 2011 [12]; El-Bakary and Sayed, 2011 [8]; Treguier *et al.*, 2011 [13]; Celi *et al.*, 2013 [14]; Chen *et al*., 2013 [15]; Desouky *et al*., 2013 [7]; Ding *et al.*, 2013 [16]; Meng *et al*., 2013 [17]; Sepici-Dincel *et al*., 2013; Xu *et al.*, 2013 [18]; Garzoli *et al.*, 2014 [19]). *P. clarkii* is very adaptable physiologically and behaviourally (Stanton, 2004 [20] and El-Bakary and Sayed, 2011 [8]). Nowadays, there is an important crayfish industry focused on the use of red crayfish as a high quality food product (Romero *et al*., 2011) [21]. This crayfish is one of the important economic aquatic crustacean species. Generally, *P. clarkii* has great resistance to diseases in the natural environment (Meng *et al.*, 2013) [17]. Histological changes have been widely used as biomarkers, both in the laboratory and field studies (Sayed *et al.*, 2015) [22]. The gastrointestinal tract of crayfish is a relatively simple and a straight tube. The gastrointestinal tract is generally considered not to act as a site of excretion of nutrients of endogenous origin (Brown, 1995) [23].

Sunlight is a key environmental factor in almost all ecosystems, and the many ecological effects of visible and infrared wavelengths have long been known (Paul and Gwynn-Jones, 2003) [24]. The alteration induced by extreme photoperiods or light intensities on the biological rhythms of diverse animals is well known and suggests the importance of this environmental parameter. Crayfish activity is regulated accordingly by the presence or absence of light and moult and reproduction seem to be photoperiodically regulated (Fanjul-Moles *et al.*, 1998) [25]. The increased incidence of UV radiation on the Earth's surface is receiving more attention because it can produce biological changes and some impact on biodiversity (Mckenzie *et al.*, 2007) [26]. Ultraviolet (UV) radiation is conventionally divided into three wavebands: UV-C (200–280 nm), UV-B (280–315 nm) and UV-A (315– 400 nm) (Paul and Gwynn-Jones, 2003) [24]. Several methods for the assessment of UV damage have been described including physiological, morphological, and biochemical studies on vertebrates and invertebrates (El-Bakary and Sayed, 2011) [8]. However, additional studies are necessary in order to understand the responses of biological systems to UV radiation damage. The present study was designed to evaluate the effects of UV-A irradiation on the intestine of the crayfish *Procambarus clarkii*.

2-Materials and methods

2-1-Specimens collection: Mature male crayfish *Procambarus clarkii* were collected from the River Nile at El-Wassta island, Assiut Governorate, Egypt.

2-2- Preparation of Specimens for study: Specimens were transported to the laboratory and reared in aerated aquaria in the ambient temperature for two weeks for adaptation. Each aquarium was about 100 L in size and provided with clean gravel to match the natural habitat. Specimens were kept away from direct sunlight. The water depth in the aquaria was about two to three centimeters. The water volume equal (30cm×60cm×3cm=5400 cm^3 or 5.4 Liter). Tap freshwater was added from time to time to keep the level of the water in the aquaria. Before adding the water, it was left for about 24-48 hours to get rid of the chlorine. The water was changed if it becomes contaminated with a cloudy appearance or unpleasant odor. Specimens were fed daily with commercial pellets of artificial fish food; the ideal size portion of food for the crayfish is about the size of a kernel of corn. Feeding the crayfish once or twice a week was sufficient. Crayfish were placed in three groups: Control group (un irradiated group), UVR-A treated group exposed for 15 min./day for two weeks, UVR-A treated group exposed for 30 min./day for two weeks, each group consisted of six animals, each treated group was lifted two weeks after treatment for recovery.

2-3- UV-A source: The Crayfish was exposed to UV-A using UV lamp of 366nm peak transmission. The UV-A source fitted 20 cm above the experimental aquaria.

2-4-Histological preparations

After dissection of the animal, a fixed middle part of the hind gut was removed for histological preparations.

2.4.1. For light microscopic examinations: The tissues were fixed and prepared through the routine technique according to Drury and Wallington (1980) [27]. The intestine was fixed in Khale's solution for 48 hours, followed by washing in 70% ethanol for 24 hours; tissues were left in 70% for preservation until used. Dehydration in upgrade of alcohols cleared in xylene and mounted in DPX.

2.4.2. For transmission electron microscopic examination: Ultrathin sections from the selected areas of the trimmed blocks were made and collected in copper grids. The ultra-thin sections were contrasted in uranyl acetate for 10 min, lead citrate for 5 min and examined under the transmission electron microscope model JEM, 100 cx11 TEM, at Assiut University.

2-5- Histochemical studies:

2.5.1. Periodic Acid Schiff's (PAS) reaction: The periodic acid Schiff's technique (PAS) of Hotchkiss (1948) [28] was applied for carbohydrate demonstration. Fixation was carried out in Khals solution. Deparaffinized hydrated sections were placed in 1% periodic acid for 5 minutes, washed and then treated with Schiff's reagent for 20 minutes. Sections were then transferred through freshly prepared 0.5% sodium bisulphite for 3 changes, followed by washing in running tap water, dehydraed in ethyl alcohol, cleared in xylene and mounted in DPX. The PAS-positive material appears pink.

2.5.2. Bromo phenol-blue: 100 mg of bromophenol blue was mixed with 10 ml of distilled H2O then stored at room temperature. Sections were immersed in 95% alcohol, stained for 5 min and treated in 0.5% acetic acid for 5-7 minutes. Specimens were transferred in running water for 3 min, then dehydrated in series of 95%-100% ethanol and rinsed in xylene (Drury and Wallington, 1980) [27].

3-Results

(1) Light microscopic examinations:

a- Control state:

Light microscopic examinations of untreated crayfish intestine showed that there are two types of striated muscles lining the intestine from outside, outer thin layer of circular muscles and inner thick layer of longitudinal muscles (Fig. 1 a). The longitudinal muscle layer is thicker than the circular layer. The longitudinal muscles are arranged in bundles. They occurred in the sub epithelial connective tissue between the bladder cells and the adluminal epithelium. They consisted of many strips and are multinucleate (Fig. 1 b). The intestine bears several thick longitudinal folds, each ridge or fold consists of one layer of simple columnar epithelium covered with thick layer of tetra laminar cuticle (Fig. 1 a). The columnar cells at the tops of folds are tall while the cells between the ridges are smaller with a thin layer of cuticle. The columnar cells have basal oval nuclei restricted on the basal cytoplasm. At the bases of folds the nuclei of the epithelial cells are rounded with condensed chromatin and they are deeply basophilic, some with prominent nucleoli. The epithelial layer contains many goblet cells (Fig. 1 a). Beneath the epithelium, a sub epithelial connective tissue layer contains numerous bladder cells giving the connective tissue the net appearance (Fig. 1 a). The bladder cells are vacuolated cells with large central ovoid nuclei. Two types of glands can be observed; mucus glands and serous glands embedded in this layer (Fig. 1 a).

The longitudinal muscles which are embedded in the connective tissue covered with a thin layer of this tissue that binds the longitudinal muscles together (Fig. 1 b).

There is no conspicuous basement membrane at the epithelium–connective tissue interface (Fig. 1 b). The ducts of the glands that placed in the connective tissue of intestinal ridges are small. There are no obvious openings of these ducts in the lumen. The nucleus of the mucus gland is larger than that of serous gland. Pale secretion appears in the cytoplasm of mucous gland (Fig. 1 a).

b- Treated crayfish:

After 15 minutes of UV-A irradiation, some epithelial cells showed phagocytic activity which may ingest necrotic cells (Fig. 1 c). The cuticular layer surrounding the epithelial tissue showed clear separation of the under laying adluminal epithelium, also epithelial inflammation and filtration were observed (Fig. 1 c). Increased vacuolation and infiltration in the sub epithelial connective tissue were noticed (Fig. 1 c). Many histological changes in the intestinal muscle layers including: rupture in the circular muscle layer, separation of longitudinal muscle bundles from each other with fragmented and damaged muscle fibres (Fig. 1 d).

After 30 minutes of UV-A irradiation, the cuticle is separated from the underlying epithelium, showing signs of degeneration and fragmentation (Fig. 1e). Also, irregular structure of epithelial layer with abnormal appearance of columnar cells can be observed. The nuclei of the epithelial cells became hypertrophied with intense basophilic stain while some epithelial cells showed signs of mitotic cell division. The connective tissues under laying the epithelial cells showed signs of tissue necrosis and large vacuoles (Fig. 1 e). Obvious differences in muscular layer were recorded including: irregular structure of circular muscle layer. The longitudinal muscle bundles are greatly separated from each other. Shrinkage of muscle bundle with atrophic and fragmented muscle fibres is also observed (Fig. 1 f).

c- Recovered groups for two weeks:

Recovered group after irradiation for 15 min. showed that the cuticle appears thin especially between intestinal ridges where it acquires pale staining while the rest of the cuticle appears differentiated into layers with different staining affinity. The intestinal epithelial nuclei have irregular shape and arrangement with the appearance of mitotic cell division and the nuclei are larger than that of non-irradiated sections (Fig. 1 g).

Many haemocytes were observed between the epithelial cells as well as in connective tissue layer (Fig. 1 g). The density of longitudinal muscle fibres is still less than that of non-irradiated animals (Fig. 1 h).

The interstitial connective tissue between different bundles reduced compared with irradiated animals (Figs. 1 g & 1 h). The circular muscle layer acquires normal appearance (Fig. 1 h).

Recovered group after irradiation for 30 min. showed that, the cuticle appears irregular with fragmented different layers. The epithelial tissue layer shows some vacuolization and separation from connective tissue layer (Fig. 1 i). The connective tissue layer is largely vacuolated with the appearance of haemocyte infiltration. The nuclei are larger than those of non-irradiated sections (Fig. 1 i). The density of muscle fibres is still reduced compared with non-irradiated (Fig. 1 j).

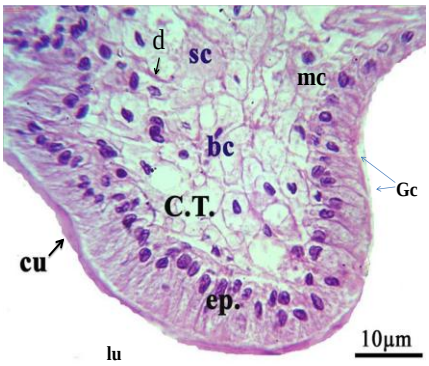
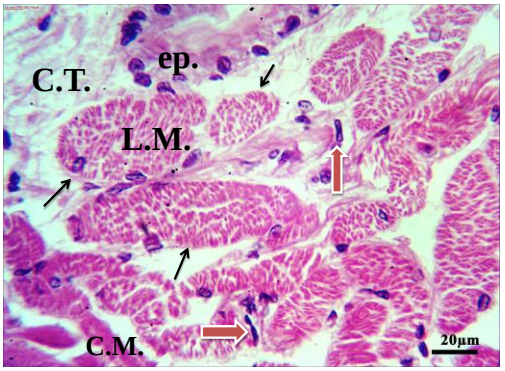


Fig. 1 (a)



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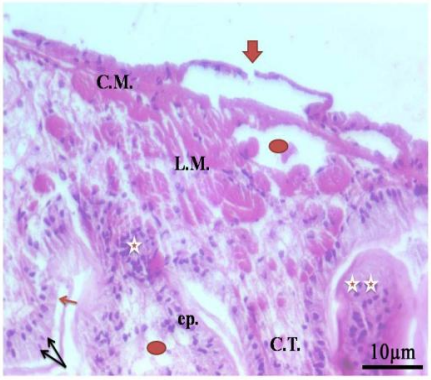

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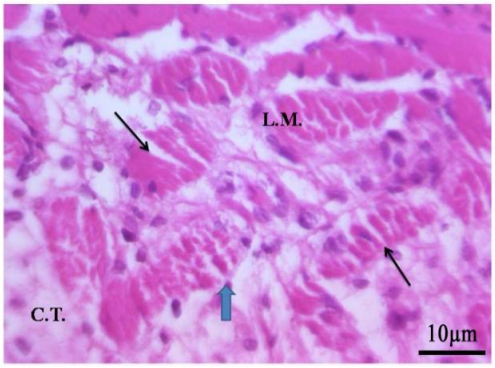

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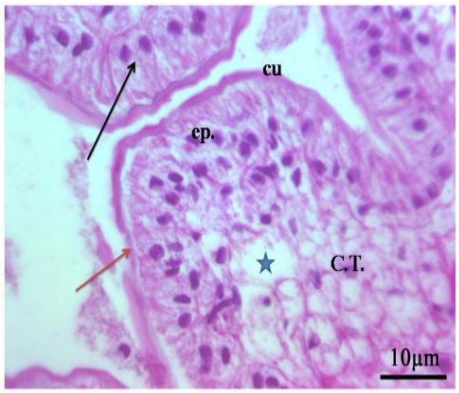

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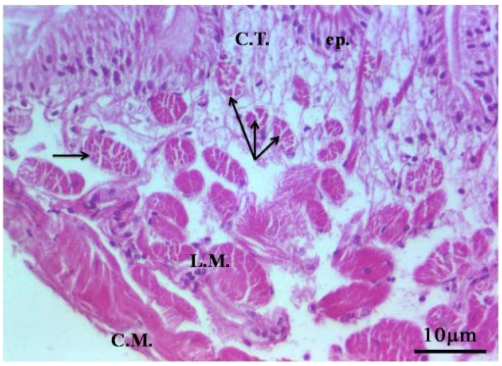

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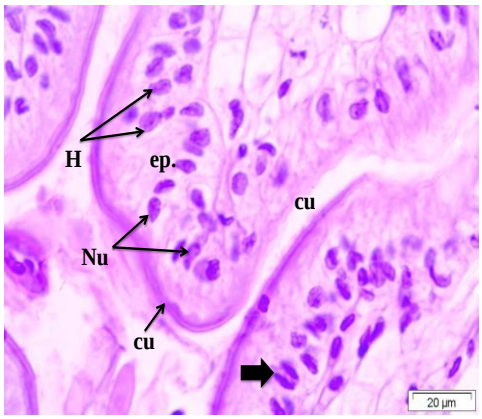

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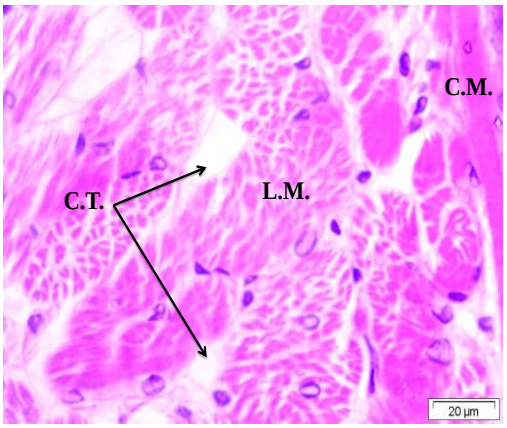

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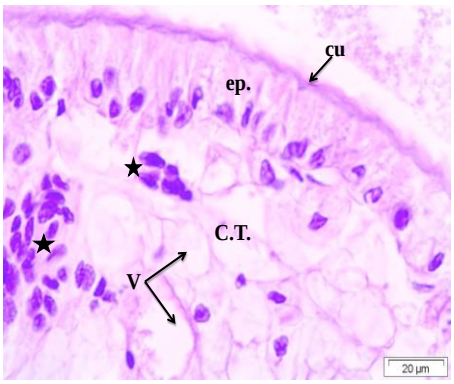


Fig. 1 (i)

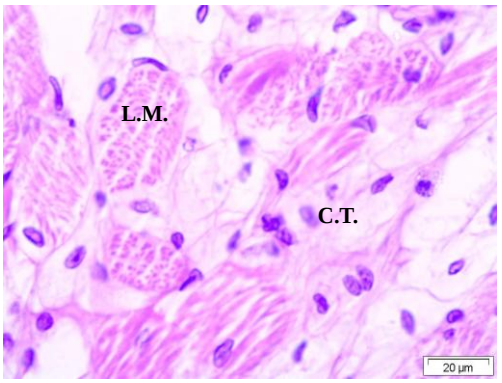


Fig. 1 (j)

Figure (1): (a-j) light micrographs: (a) control (untreated) intestine of *Procambarus clarkii* showing cuticle (cu) covered epithelial layer (ep.), connective tissue (C.T.) which contains numerous bladder cells (bc), mucus gland (mc), serous gland (sc) with its duct (d), goblet cells (Gc) and lumen (lu)- (b) muscular layer of untreated crayfish showing circular muscle layer (C.M.) and longitudinal muscle layer (L.M.)- (c) treated crayfish for 15 min. showing circular muscle (C.M.), connective tissue (C.T.), epithelial layer (ep.), inflammation(double star), infiltration (star), separation of cuticle from epithelium (arrow), phagocytic cells ingesting necrotic cells (double arrows), rupture of circular muscle (thick arrow) and vaculation (circles)- (d) damaged (arrows) of longitudinal muscle layer (L.M.)- (e) treated crayfish for 30 min. showing necrosis in connective tissue (star), damaged of cuticle (red arrow) and hypertrophied and intensely basophilic nuclei (black arrow)- (f) damaged in longitudinal (L.M.) and circular (C.M.) muscle layers- (g) recovered crayfish after 15 min. of irradiation showing hemocytes (H) in epithelium (ep.), large nuclei (Nu) and mitotic division(thick arrow)- (h) incomplete recovered in longitudinal muscle layer (L.M.)- (i) recovered crayfish after 30 min. of irradiation showing hemocyte infiltration (star), vacuolation of connective tissue (v) and irregular cuticle (cu)- (j) damaged unrecovered longitudinal muscle layer (L.M.).

(2) Histochemical study:

Application of periodic acid Schiff's technique (PAS):

a- Control state: the cuticular layer of the intestinal epithelium is strongly stained (+++positive reaction), the intensity of the stain is reduced between folds of intestinal ridges (Fig. 2 a). In the case of epithelial cells, the cytoplasm as well as the epithelial membranes are faintly stained (Fig. 2 a). The connective tissue is faintly stained with the exception of some areas where glands are localized. The muscle layers (circular and longitudinal) are faintly stained while the sarcolemma of these fibres is strongly stained (Fig. 2 b).

b- Treated crayfish:

After 15 min. of irradiation, the intensity of PAS stain of cuticular layer is similar to control group, while the epithelium and the connective tissue layer showed strong PAS positive (Fig. 2 c). The longitudinal muscle layer showed colour difference in these bundles (Fig. 2 d).

After 30 min. of irradiation, the cuticular regions which are separated from the underlying epithelium appear faintly stained while the regions of damaged and fragmented cuticle give negative reaction (Fig. 2 e). The epithelial cell cytoplasm are faintly stained but the damaged cells in the epithelium gave negative PAS reaction (-ve) (Fig. 2 e). The connective tissue is faintly stained compared with non-irradiated sections. Circular and longitudinal muscle layers acquire rather faint staining when compared with non-irradiated sections (Fig. 2 f).

c- Recovered groups after two weeks:

Recovered group after irradiation for 15 min. showed that the intensity of PAS in the cuticular layer is reduced when compared with non-irradiated sections (Fig. 2 g). Between intestinal ridges the cuticle is faintly stained (Fig. 2 g). The intestinal epithelium and connective tissue are also faintly stained except the regions where glands are present (Fig. 2 g). In case of longitudinal and circular muscles, they are strongly stained (Fig. 2 h).

Recovered group after irradiation for 30 min., the cuticle appears irregular with fragmented different layers (Fig. 2 i). The epithelial tissue layer shows some vacuolization and separation of connective tissue layer. The connective tissue layer is largely vacuolated with the appearance of haemocyte infiltration. The nuclei are larger than those of non-irradiated sections (Fig. 2 i). The interstitial connective tissue between longitudinal muscle bundles is larger when compared with previous group. The density of muscle fibres still reduced when compared with non-irradiated one (Fig. 2 j).

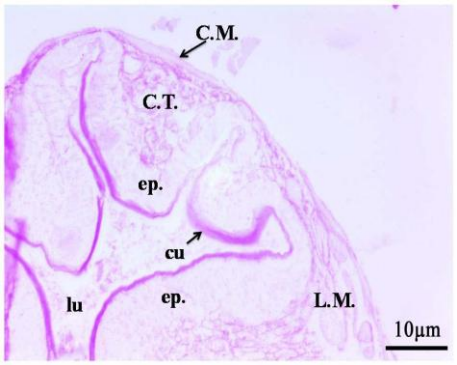
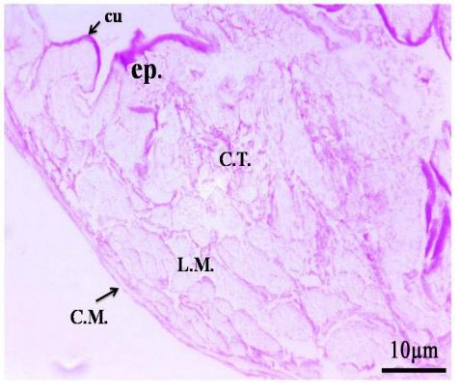


Fig. 2 (a)



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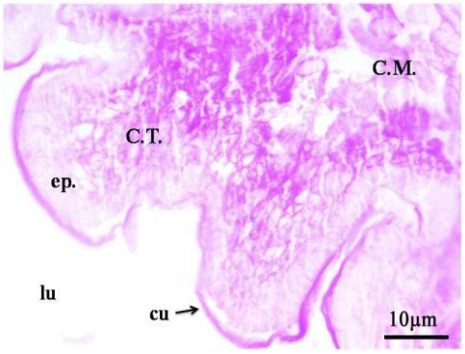

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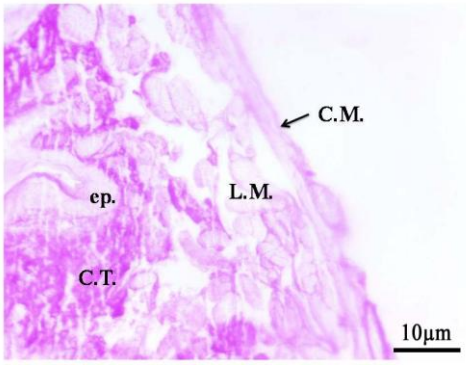

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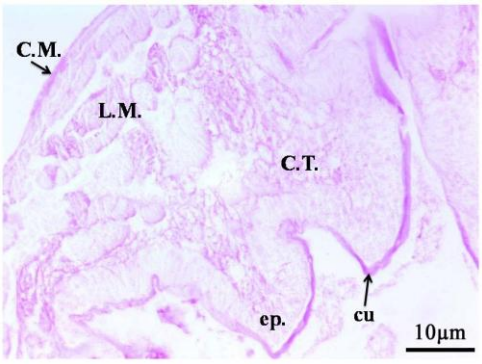

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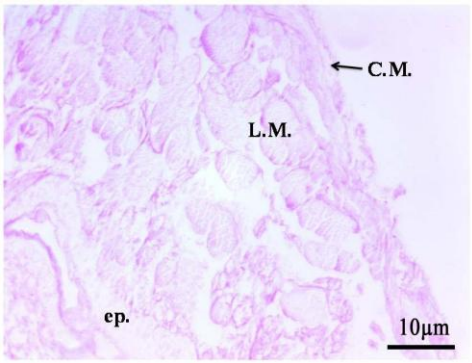

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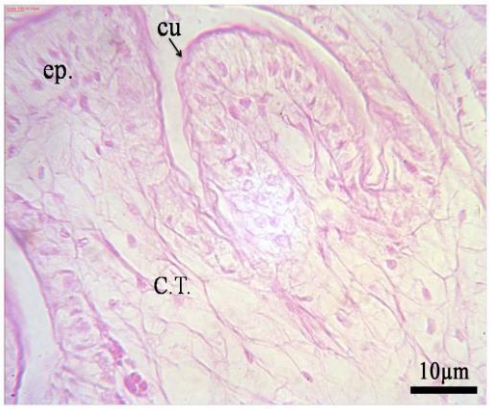

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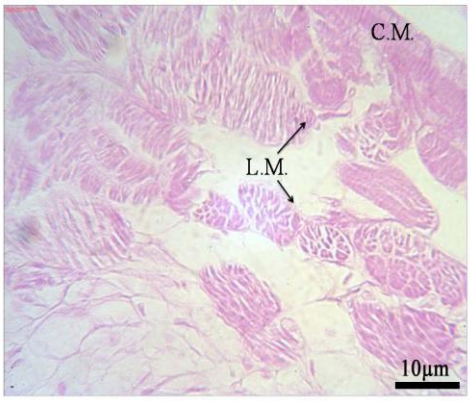

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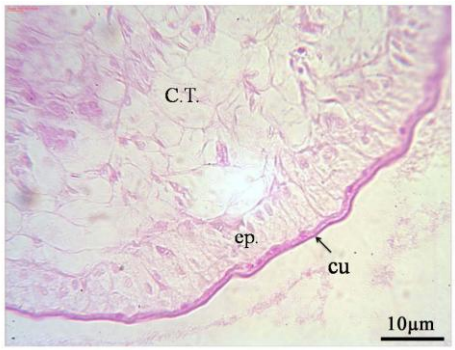


Fig. 2 (i)

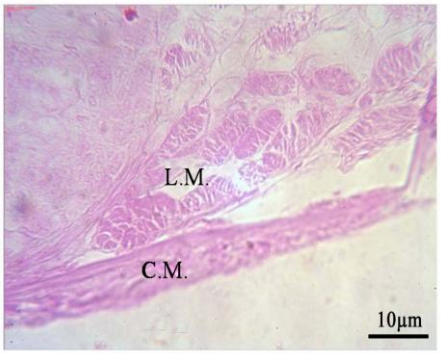


Fig. 2 (j)

Figure (2): (a-j) light micrograph of intestine stained with periodic acid Schiff technique showing the reaction of this stain in untreated crayfish(a,b), treated crayfish for 15 min.(c,d), treated crayfish for 30 min.(e,f), recovered group after 15 min. of irradiation (g,h) and recovered group after 30 min. of irradiation (i,j).

Application of bromophenol blue technique:

a- Control state: cuticle, intestinal cells and their nuclei are strongly stained (+++ve) (Fig. 3 a). The cuticle which was located between folds is faintly stained. The borders of sub epithelial connective tissue (bladder cells) are strongly stained while their interior are faintly stained. The connective tissue glands showed strong affinity to bromophenol blue stain (Fig. 3 a). The longitudinal and circular muscle layers are strongly stained (Fig. 3 b).

b- Treated crayfish:

After 15 min. of irradiation, weak reaction of bromophenol blue staining was observed especially in the cytoplasm of the adluminal epithelium with appearance of a weak metachromatic shadow in the cytoplasm (Fig. 3 c). The nuclei of the epithelial cells had the same staining affinity as in untreated group. The regions where the glands, the borders of bladder cells and their nuclei are faintly stained give weak reaction with bromophenol blue stain (Fig. 3 c). In case of longitudinal muscle layers: some of these bundles give strong reaction but the others give weak reaction (Fig. 3 d). Circular muscle layer gives strong reaction (Fig. 3 d). The cuticular layer surrounds the epithelial tissue showed clear separation of the under laying adluminal epithelium, some regions of the cuticle stained faintly while small areas showed positive reaction (++ve). Histochemical changes were observed in the intestinal muscle layers where the longitudinal muscle bundles showed a positive weak reaction compared with circular muscle layer.

After 30 min. of irradiation, cuticular layer, adluminal epithelium, connective tissue and muscle layers give weak reactions (Figs. 3 e&f). The cuticular layer and epithelium showed magenta colour (Fig. 3 e). The glands showed negative reaction to bromophenol blue stain (Fig. 3 e).

c- Recovered groups for two weeks:

In recovered group after irradiation for 15 min., the cuticular layer of intestinal epithelium is faintly stained when compared with non-irradiated ones with the appearance of magenta colour in one of the tetra laminar layer of cuticle (Fig. 3 g). The cytoplasm of intestinal cells is faintly stained but the nuclei of these cells are strongly stained. Connective tissue is faintly stained except the regions of glands (Fig. 3 g). Longitudinal and circular muscles are strongly stained (Fig. 3 h).

Recovered group after irradiation for 30 min. showed that, the cuticle of intestinal cells is faintly stained when compared with control group (Fig. 3 i). The cytoplasm and nuclei of intestinal cells showed stronger affinity when compared with irradiated group. Connective tissue is faintly stained except the borders of bladder cells and glands while muscle layers (longitudinal and circular) are strongly stained (Fig. 3 j).

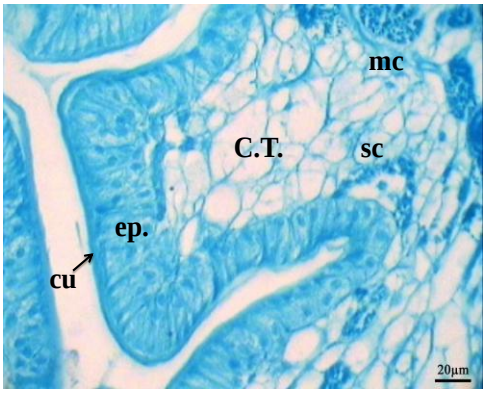


Fig. 3 (a)

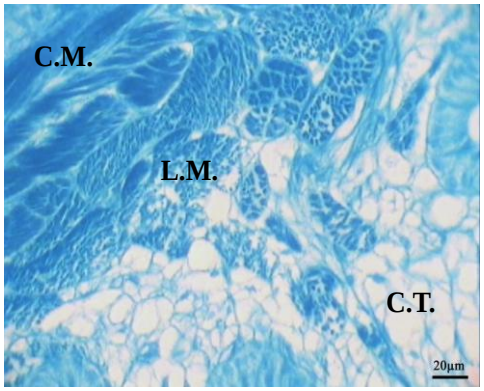


Fig. 3 (b)

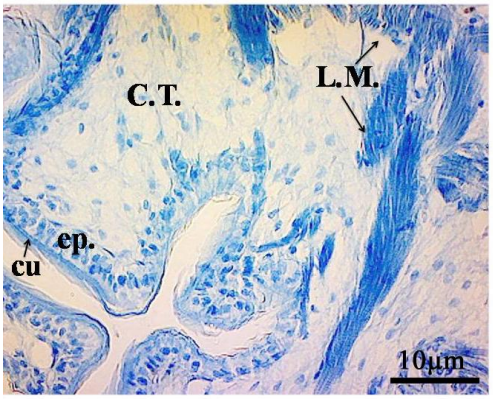


Fig. 3 (c)

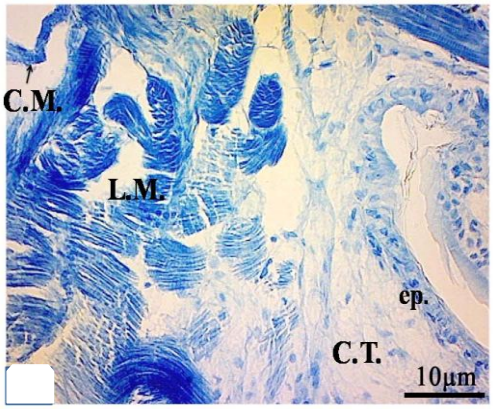
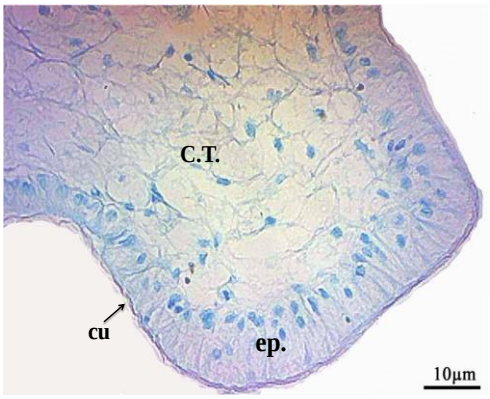


Fig. 3 (d)



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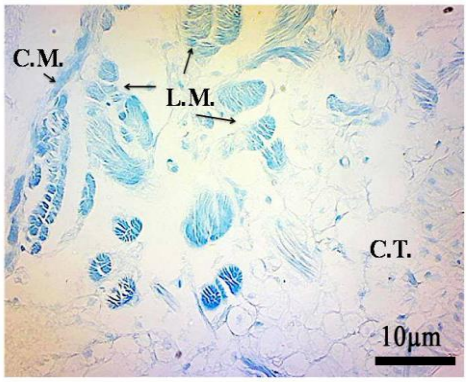

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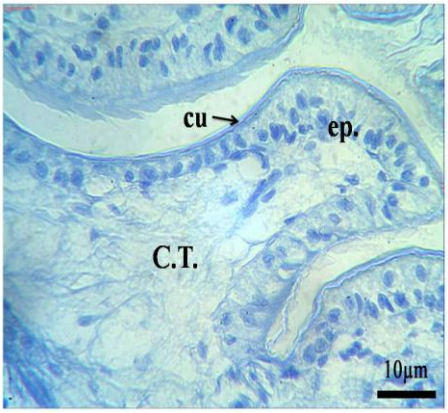

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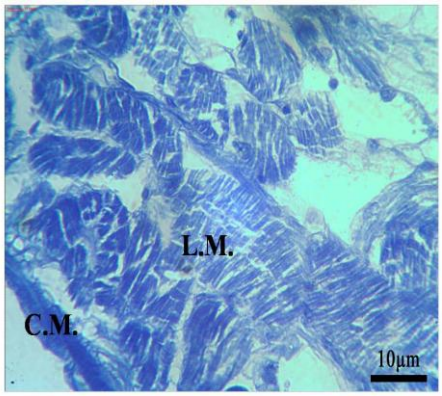

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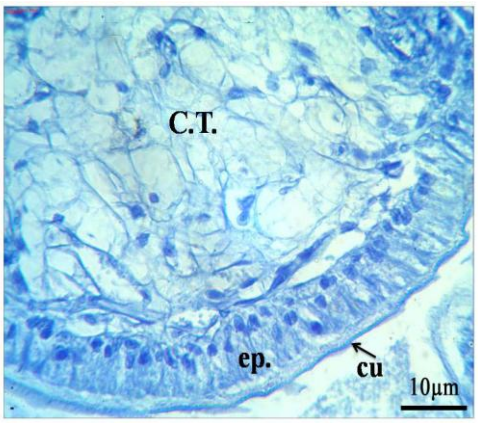

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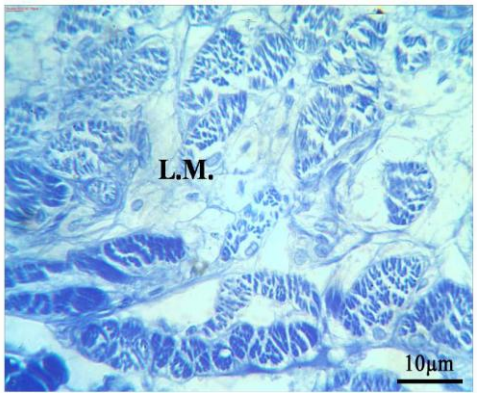


Fig. 3 (j)

Figure (3): (a-j) light micrograph of intestine stained with bromophenol blue technique showing the reaction and intensity of this stain in untreated (a,b), treated crayfish for 15 min. (c,d), treated crayfish for 30 min. (e,f), recovered crayfish after 15 min. of irradiation (g,h) and recovered crayfish after 30 min. of irradiation (i,j).

(3) Electron microscopic study:

a- Control state: The apical cell membrane of the epithelial cells is generally infolded (Figs. 4 a&b). The basal cell membranes are elaborately infolded. Zonula adherens and septate desmosomes are common cell-to-cell junction types (Fig. 4 c). The lateral cell membrane in the junctional region is moderately to extensively interdigitated. Micropinocytosis vesicles are seen in process of invagination (Fig. 4 b). The cytoplasm of the epithelial layer is full of many organoids. The cytoplasm in the apical region of the cells possessing numerous mitochondria which indicated that these cells are very active (Figs. 4 a&b). The cytoplasm also contains endoplasmic reticulum (smooth and rough) (Fig. 4 b), lipid droplets (Fig. 4 c) and myelin figure (Fig. 4 a). The nuclei of the epithelial cells are multilobed with condensed chromatin (Fig. 4 a). The multilobed structure of the nucleus leads to a significance increase in the surface area of the nucleus. The nuclei of epithelial layer are limited by a bilaminar nuclear envelope with two types of chromatin, the condensed and stainable form, heterochromatin, and the dispersed chromatin in the nuclear matrix, euchromatin (Figs. 4 a&b). They contain large amounts of heterochromatin and small amounts of euchromatin (Figs. 4 a&b). The heterochromatin typically is dispersed in the nuclei with small islands of euchromatin in between (Figs. 4 a&b). No apparent nucleoli were observed in the different tissue sections. Nerve cell with axons were also observed and surrounded by myelin sheath (Fig. 4 d).

b- Treated crayfish:

After 15 min. of irradiation, epithelial layer was damaged, the cell membrane appeared thin with the destruction of inter epithelial junction (Fig. 4 e). The cytoplasm showed vacuoles with no clear organoides while the nuclei appeared shrinkage with condensed chromatin at the periphery of the nuclear membrane (Fig. 4 e).

After 30 min. of irradiation, the ultra structure sections of the intestinal epithelium showed fragmentation of the cell membrane which appears as dark dots (Fig. 4 f). The cytoplasm is totally vacuolated while the shrinked nuclei with condensed chromatin at the periphery of the nuclear membrane (Fig. 4 f).

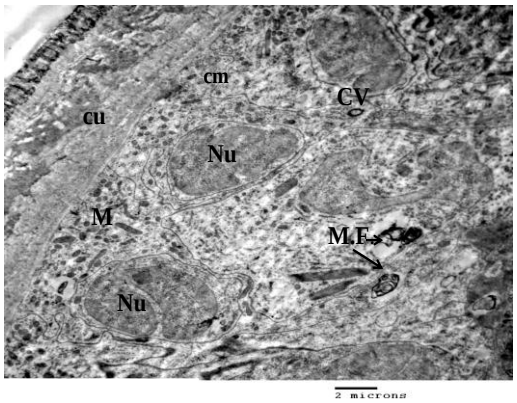


Fig. 4 (a)

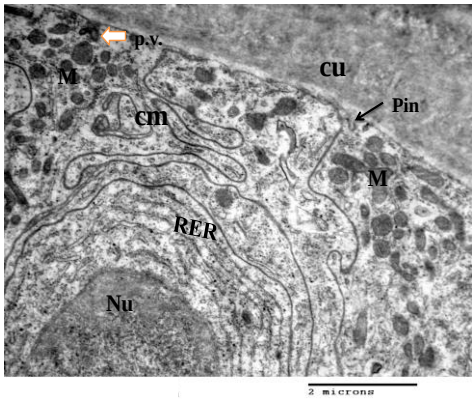


Fig. 4 (b)

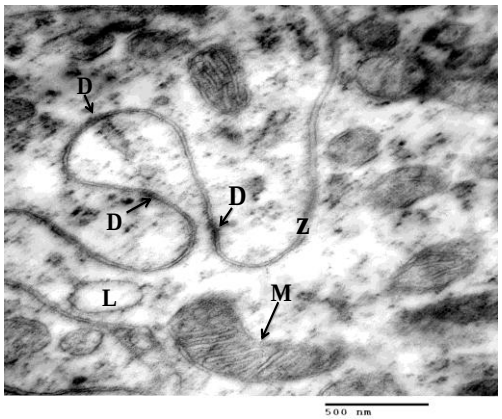
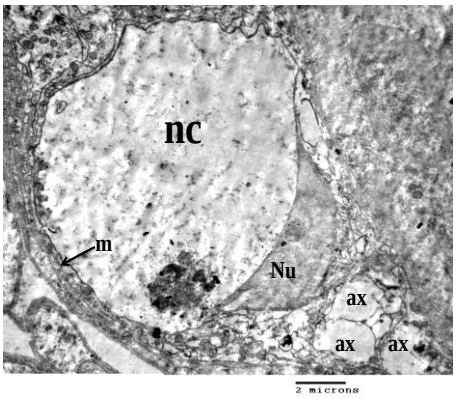


Fig. 4 (c)



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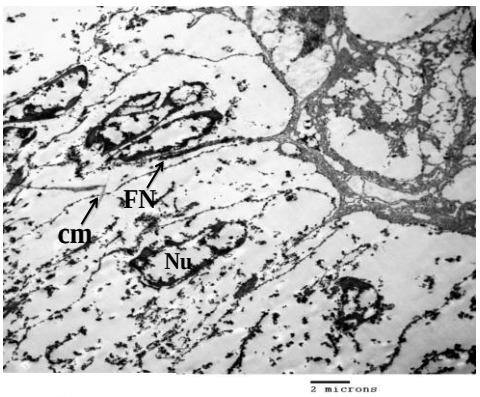

Fig. 4 (e)

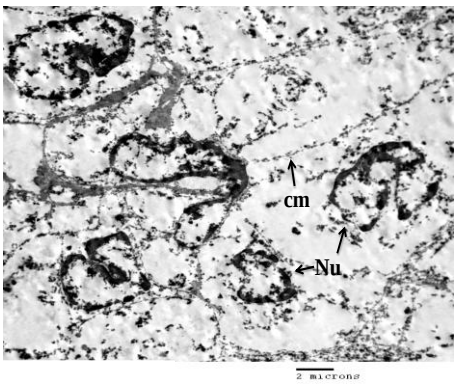

Fig. 4 (f)

Figure (4): (a-f) electron micrograph of intestine of crayfish in untreated group (a,b,c and d) showing infolded cell membrane (cm), cuticle(cu), coated vesicle(cv), mitochondria(M), myline figure (M.F.), nucleus (Nu), rough endoplasmic reticulum(RER), pinocytotic vesicle (pin), desmosomes (D), lipid droplet (L), axons (a) of nerve cell (nc), myline sheath (m), (e) treated crayfish for 15 min. showing fragmented nucleus (FN) and (f) treated crayfish for 30 min. showing fragmented cell membrane (cm) and fragmented nucleus(FN).

4-Disscusion

Crayfish has been widely used as a bioindicator for detecting the effects of environmental changes (El-Bakary and Sayed, 2011) [8]. The alimentary tract of decapod crustacean carries out a number of physiological functions including digestion, absorption, storage and transport of materials, both foregut and hindgut have been implicated in ion and water movement (Mykles, 1979) [29].

The present study revealed that, the intestine of *Procambarus clarkii* has inner longitudinal and outer circular bands of striated muscles. The circular muscle layer is thinner than that of the longitudinal muscle which together generates peristaltic contractions that move faecal string down to the alimentary canal for expulsion. These observations are similar to that of Winlow and Laverack (1972) [30]; Factor (1995) [31]; Brenner and Wilkens (2001) [32]; and To *et al*. (2000) [33]. In contrast, El-Bakary and El-Gammal (2010) [34] demonstrated that in fishes there is a thick inner circular layer and a thinner outer longitudinal layer which may reflect a different mechanism for peristaltic movement. Thus, the intestinal muscles of *Procambarus clarkii* are specialized to produce slow peristaltic waves of contraction. Brenner and Wilkens (2001) [32] showed that vertebrate visceral smooth muscles not unlike those seen in this study, the contraction of longitudinal muscle layer shorten the intestine while circular muscle layer cause tighten of the intestine leading to the expelling of the faeces. Chisaka *et al.* (1999) [35] showed that the circular muscle layer in the intestine pulls the cuticular layer to expand the hindgut lumen. According to Winlow and Laverack (1972) [30] the longitudinal muscle strips were found to beat independently of one another, the independent rhythmicity of longitudinal and circular muscles suggest that spontaneous hind gut motility occur in the lobster *Homarus gammarus*.

Mercier and Lee (2002) [36] working on crayfish and Winlow and Laverack (1972) [30] working on lobster deduced that during spontaneous movement, contractions of the circular muscles are typically much slower than those of the longitudinal muscles which contracted more rapidly. As in other species, peristaltic movement of the intestines of decapod crustaceans involves coordinated contractions of circular and longitudinal muscles. This conclusion may be attributed to circular muscle layer in this study which is much thinner than longitudinal muscle layer. The control of these peristaltic movements is a subject of controversy, according to Wales (1982) [37]; Brown (1995) and Mercier and Lee (2002) [36] central nervous system is responsible for this control while, Shuranova *et al*. (2003) [38] mentioned that peristaltic contractions are controlled via endogenous pacemakers without neural input. Comparative investigations made on different invertebrates by Prosser *et al*. (1965) [39] are in favour of a nervous mechanism of spontaneous activity in crustacean intestinal muscles. In the present investigation, the presence of nerve cells and axons in the intestine demonstrate without confusion that the hind gut of *P. clarkii* is under control of central nervous system.

In the present study, two types of glandular cells (mucus glands and serous glands) in the sub epithelial connective tissue of the intestine of *P. clarkii* are easily distinguished under the light-microscope. The same observations were recorded by To *et al*. (2004) [33]. The present investigation showed that these glands contained neutral polysaccharides and proteins. The mucous cells are implicated in the production of mucus for packaging and lubrication of faeces. According to Barker and Gibson (1978) [40]; Felder and Felgenhauer (1993) [41] and To *et al.* (2004) [33] these glands are possibly responsible for the elaboration of a peritrophic membrane around the luminal contents.

The present study revealed that the main intestinal tissue project in the intestinal lumen consists of ridges; each one is composed of an adluminal epithelium with a dense irregular connective tissue. The present study showed that in *P. clarkii*, adluminal epithelium composed of columnar cells which have oval basal nuclei with condensed chromatin, there is no conspicuous basement membrane at the epithelium–connective tissue interface. Same observation was recorded by To *et al*. (2004) and Sousa and Petriella (2006) [42]. The latter described the hindgut of *Palaemonetes argentinus* which resembles that of *P. clarkii*.

In *P. clarkii*, a cuticular layer covering the luminal surface is present, according to Luquet *et al*. (2013) [43], the epithelial cells of the foregut in Decapoda are responsible for the synthesis of the cuticle. The present study, using electron microscope showed that the cuticular layer appears dense with different layers. The cuticle size and colour intensity are due to the amount of resin as appear by ultrastructure technique. The hindguts of different crustaceans also have a cuticular layer on apical surface of the adluminal epithelium as appeared in different species like, *Homarus americanus*, *Homarus gammarus*, *Cancer magister*, *Callinectes sapidus*, *Portunus sanguinolentus* and *Lepidophthalmus louisianensis* (To *et al*., 2004). In other crustaceans, the entire gut is lined by a cuticle indicating its completely ectodermal origin as in *Cyathura carinata* (Wägele *et al*., 1981) [44]. This cuticle affords protection of soft tissues from abrasion by the luminal contents (Factor 1979 [45]& 1995 [31]; Mykles 1979 [29]; Johnson 1980 [46]; Trinadha Babu *et al*. 1989 [47] and Felder and Felgenhauer 1993 [41] & To *et al.,* 2004 [33]).

Histochemical reactions of the present study indicated that, in case of periodic acid application (PAS), the cuticular layer of the adluminal epithelium gave strongly PAS positive reaction, while the epithelium, connective tissue and muscular layers gave weak PAS positive reaction which indicated that the cuticle contains high amounts of mucosubstances. This may indicate absorption of neutral polysaccharides directly from feeding and/or release from it. El-Bakary and El-Gammal (2010) [34] showed different mucosubstances correlated with assorted digestive function in fish. The presence of these macromolecules has been mentioned in several decapod species, and they seem not to be associated with digestive processes (Lovett and Felder, 1990 [48]; Johnston and Alexander, 1999 [49]). According to Mary & Krishnan (1974) [50]; Malley (1977) [51]; Mykles (1979) [29] and Johnson (1980) [46] Sousa and Petriella, (2006) [42], the cuticles in decapod crustaceans are permeable to water and salts and they may allow the transport of both water and ions. In *P. clarkii*, the presence of mucosubstances protects the epithelial surface and forms a resistant barrier. In general, it is known that the mucus secretion acts as lubricant and as a defence mechanism against several toxic substances. The mucopolysaccharides detected in this study on the cuticle, glands and lumen may lubricate the indigestible food bolus as it passes towards the hindgut.

Bromophenol blue application is a biological stain which can be used to stain proteins and nucleic acids. The present study revealed that Bromophenol blue stain reacts positively with the absorptive columnar cells of the epithelial folds, such a positive staining product was present in the place of the chromatin substances containing DNA, which mean that the epithelial cell layer may play a role in synthesis and/or storage and secretion. The functions of the different glands, serous gland with its weak staining reaction and mucus gland with high protein staining cells cannot be explained in detail without data on the enzymatic activity of their secretions, provided the role and nature of their physiological function. Electron microscopic observations showed that the cytoplasm of these cells contains many organoids, the apical membranes of these cells are infolded with a great numbers of mitochondria. The presence of mitochondria suggests the occurrence of energy requiring processes. In the present study, the presence of pinocytotic vesicles suggests an absorptive role of the intestine. The apical membrane of these cells often exhibits considerable infolding into the cytoplasm with extensive rough and smooth endoplasmic reticulum which reveals that these cells are also concerned with synthesis of protein, lipid and carbohydrate. The infoldings may enable the hindgut lining to stretch under large loads and thus contribute to the elasticity of the lining (Komuro and Yamamoto, 1968) [52]. Thus the epithelial cells of the intestine of *P. clarkii* indicated that they may play a role in resorption of food and secretion of digestive enzymes. According to Talbot *et al.* (1972) [53]; Mykles (1979) [29] & Ahearn (1988) [54], the cytoplasm of intestinal epithelium of decapods has tubular smooth endoplasmic reticulum which is apparently continuous with the basal plasma membrane. The cells of the intestine of *P. clarkii* are interconnected by Zonula adherens and septate desmosomes which are common cell-to-cell junction types. The lateral cell membrane in the junctional region is moderately to extensively interdigitated. Gap junction provides a mechanism for slow transmission of excitatory depolarization appropriate for the generation of peristaltic contractions (Fawcett, 1981) [55].

In the present study, after 15 min. of UV-A irradiation, the breakdown of circular and longitudinal muscles fibre layers of the intestine was evident, connective tissue which binding these muscles was vacuolated. According to Sayed *et al.* (2007) [56], the UV-radiation had great significant impacts on the treated fish in comparison with the control ones. UV-radiation has severe impacts on the aquatic animals including fish, zooplankton and phytoplanktons, the mechanism of action of UV radiation on these animals was found to be different from that of other pollutants such as heavy metals and pesticides (Mahmoud *et al.,* 2009) [57].

In the current study, the adluminal epithelium shows phagocytosis with separated cuticle. According to Pazir *et al.* (2012) [58], the hypertrophied columnar cells and intranuclear inclusion bodies were observed in midgut of shrimp farms, *Litopenaeus vannamei*, after the infection of white spot virus, and some cells were ruptured and released inclusion bodies to the lumen of the gut.

The present study revealed that, in treated groups, the histochemical study showed intense PAS staining of neutral polysaccharides in the connective tissue and epithelial layer after 15 min. of irradiation which indicated that the amounts of glycogen and free glycoproteins increased in the cytoplasm of these cells. The presence of increased amount of mucosubstance in 15 min. of irradiation in the epithelial and connective tissue layer is possibly to regulate the transfer of proteins, ions and water. A similar case was described in fishes by El-Bakary and El-Gammal (2010) [34]. The mucopolysaccharides detected on the epithelium and lumen may lubricate the increased indigestible food bolus passes towards the hindgut as a result of irradiation.

Weak reaction with bromophenol blue in cuticular layer, epithelial cells and connective tissue was observed after 15 min. of irradiation which indicated that the amounts of protein decreased after 15 min. of UV-irradiation. While the nuclei of epithelial cells and muscle layers gave strong reaction that may meant condensation of nucleic acids.

In the present study, using electron microscope, the irregular structure of epithelial cells with hypertrophied nuclei was observed. Also, a great damage of these cells with fragmented nuclei, a clear cytoplasm without organoids and lysis of septa between them, these signs of cell degeneration will affect the normal function.

After 30 min UV-A the atrophy of the intestinal muscle bundles as well as clear damage of the perimuscular connective tissues which binding them was observed. These results demonstrated that peristaltic contraction will be affected presuming that faecal materials will be accumulated in the intestinal lumen. The glands were not observed in 30 min. irradiated animals, which also impose the question about the function of the intestine.

In the present study, the irradiation affects epithelial cells shows signs of inflammation, infiltration of haemocytes and phagocytoses after 30 minutes of UV-irradiation. Existence of nuclear pyknosis and karyorrhexis in the intestine were accompained with losses in tissue structure.

Some reports have shown that UV induces cell apoptosis (Morita and Krutmann, 2000 [59]; Norbury and Hickson, 2001 [60]; Denning *et al.,* 2002 [61]; Zhou and Steller, 2003 [62] and Chuang *et al.,* 2006) [63]. In the present electron microscopic observations, the shrinked nuclei and vacuolated cytoplasm indicated cell apoptosis. UV triggers several apoptosis signalling pathways in many cell systems (Emeny, 2009) [64]. Separation of cuticular layer from epithelial cells and Lysis of cuticle in some areas was also observed. In *P. clarkii* intestine the appearance of mitotic figures in the epithelium seem to be a direct reflection to the destroyed cuticle which may indicate that its absence maximized the need for extensive cell proliferation and differentiation along the adluminal epithelium for its protection. Athikesavan *et al.* (2006) [65] mentioned that after 20 days of exposure of Nickel as an environmental stressor on *Hypophthalmichthys molitrix*, flattening of intestinal folds, rupture of muscular layer, reduction of villi and necrosis were prominent, in 30 days treated fish, changes, like degeneration of peritoneal lining, the loss of longitudinal, vacuolization, histolysis of columnar cells and reduction of villi were the other marked changes. Tuvikene *et al.* (1999) [66] showed that the intestine of caged and feral freshwater fish lifted the columnar cells of villi and hyperplasia as responses defence mechanism when exposed to the toxicants. UV-A irradiation for 30 min. of irradiation, verified weak PAS positive staining indicated that the amounts of mucosubstances were decreased, these results were similar to what observed in the recovery groups. This weak reaction to PAS may indicate the exhaustion and depletion of mucosubstances during the period of UV-irradiation.

After 30 min. of UV-irradiation, very weak reaction of bromophenol blue was seen in cuticular layer, epithelial cells with nuclei, connective tissue with glands and muscle layers which revealed that the increase exposure of UV-A irradiation leading to the decrease of the protein amounts in the intestine of crayfish.

In the recovered *P. clarkii,* the circular muscle layer recovered after two weeks of UV-A irradiation for 15 minutes while longitudinal muscle layer still showed sign of cell death and degeneration, the same case as in recovered animals from 30 minutes of irradiation. The unhealed connective tissues and adluminal epithelium were still clear in both groups. These observations leading to conclude those 2 weeks of recover even for short period of irradiation (15 minutes) is not sufficient time for complete recovery which suggested that the regeneration of this layer is a slowly process with a low rate of cell turnover. Because repair processes can never be completely

efficient, the less damage occurs, the greater the potential advantage to the organism, regardless of the absolute levels of repair that an organism may be able to elicit (Dahms and Lee, 2010) [67]. These results showed that two weeks of recovery of irradiated animals for 15 and 30 minutes are not enough for the healing of adluminal epithelium. In case of recovered groups, incomplete recovery was seen with signs of damage in the cuticle. In irradiated group for 15 min, the cuticle showed signs of recovery in some region, while in 30 min. irradiated group *P. clarkii* did not restore its original appearance and showed weak sign of recovery. These observations indicated that the increase time of irradiation dose need more time for recovery. Once damaged, however, the damage tends to be progressive and irreversible. The assessment of damage in various biological matrices, such as tissues and cells, is vital to understanding its consequences (El-Bakary and Sayed, 2011) [8].

In case of 15 min. recovery group, slow turnover of cuticular layer, epithelial layer and connective tissue were observed with very weak reactions with bromophenol blue stain which indicated that they couldn't retrieve their contents of proteins and they will need more time for retrieving their contents of proteins. Muscle layers gave strong reaction. After 30 min. of recovery group, epithelial layer and connective tissue gave strong reaction when compared to 30 min. irradiated group which indicated that they began to retrieve their contents of protein. These observations revealed that the intestine needs more time to retrieve its contents of protein. This study showed that the intestine of *P. clarkii* is sensitive to UV-A even for short period of irradiation (15 min. /2 weeks) and the recovery process is a slowly process.

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تأثير الأشعة الفوق بنفسجية-(أ) علي أمعاء استاكوزا المياه العذبة (بروكامبرس كلاركي)

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تعتبر استاكوزا المياه العذبة محل الدراسة من قشريات المياه العذبة والتي تنتشر في مياه نهر النيل بمصر. وأصبحت نموذج مهم في دراسة سلوك الحيوان والمؤثرات البيئية والتلوث وهذا بالاضافة الي دوره الاقتصادي. الأشعة الفوق البنفسجية لها تأثير ضار علي الكائنات المائية. وقد صممت الدراسة الحالية لتقييم تأثيرات الأشعة الفوق البنفسجية علي أمعاء استاكوزا المياه العذبة. وقد تم وضع العينات في المعمل لكي تتأقلم مع البيئه وتم تقسيمهم الي ثلاثة مجموعات. المجموعة الأولي مجموعه ضابطة لم تتعرض للأشعة والمجموعة التانية والثالثة قد تم تعريضها للأشعة الفوق بنفسجية. المجموعة الثانية تم تعريضها للأشعة الفوق بنفسجية لمدة خمسة عشر دقيقة يومياً لمدة اسبوعين والمجموعة الثالثة تم تعريضها لمدة ثلاثين دقيقة يومياً لمدة اسبوعين. وقد تم ترك كل من المجموعات التي تعرضت للأشعة الفوق البنفسجية لمدة اسبوعين للاستشفاء. وقد تم فحص دراسة وفحص أمعاء استاكوزا المياه العذبة والعضلات الطولية والدائرية التي تحيط بالأمعاء من الخارج باستخدام التقنيات الهستولوجية والهيسوكيميائية والميكرسكوب الالكتروني. وقد أظهرت نتائج هذه الدراسة وجود تدمير في تركيب الأمعاء بعد تعرضها للأشعة الفوق بنفسجية ويزداد هذا التدمير والتلف بزيادة مدة التعرض للأشعة. شمل هذا التدمير الخلايا الطلائية المبطنة للأمعاء وظهور طبقة الكيوتيكل التي تغطي الخلايا الطلائية بشكل غير طبيعي وغياب للعضيات الخلوية التي توجد داخل الخلايا والتي ظهرت بالميكرسكوب الالكتروني. وبعد ترك المجموعات التي تعرضت للأشعة لم يحدث لها استشفاء كامل.