The protective effect of *Sargassum crassifolia* against Nimbecidine –induced hepatotoxicity and nephrotoxicity in Wistar rats

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Abstract: The present work has been planned to evaluate the protective effect of *Sargassum crassifolia* against the histological and ultrastructure alterations induced in liver and kidney of male rats exposed to Nimbecidine. The rats were subdivided into four groups, control group, Nimbicidine-treated group (administered daily with 1/10 LD_{50} of Nimbicidine) and the animals in the two other groups received Nimbicidene simultaneously with 10% or 25% of algal extract. After 4 weeks of treatment, animals from each group were dissected and samples from liver and kidney were removed for histopathological and ultrastructural studies. Light and electron microscopical examination showed severe liver degeneration and mild nephropathic changes in rats treated with nimbecidine only. The hepatocytes showed necrosis, vacuolization, reduction in the rough endoplasmic reticulum and damage of mitochondria. The kidney showed damage in the glomerulus with dilatation in Bowman's capsules, destruction of cytoplasmic organelle of tubular cells and pyknotic nuclei. Treatment with *Sargassum crassifolia* improved the pathological alternations in the liver and kidney which indicates the protective role of *Sargassum crassifolia* in modulating the hepatic and renal lesions.

[Hawazin H. Mutawie and Sabah M. M. El-Naggar. **The protective effect of *Sargassum crassifolia* against Nimbecidine –induced hepatotoxicity and nephrotoxicity in Wistar rats.** *Life Sci J* 2013; 10(4): 2474-2482]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 331

Key words: *Sargassum crassifolia*, liver, kidney, Nimbecidine, toxicity, rats.

1. Introduction

Marine algae (seaweeds) have been widely used by coastal populations for thousands of years owing to their high nutritional values (MacArtain *et al*., 2007). They contain carotenoids, proteins, essential fatty acids, vitamins and minerals (Rajasulochana, *et al*. 2009). Moreover, they contain bioactive compounds like terpenoids and sulphated polysaccharides a potential natural antioxidant, which are not found in land plants (Lahaye and Kaffer, 1997). Yan *et al*. (1998) reported that, *sargassum* sp. are found to have the highest free radical scavenging property.

Polysaccharides of plant origin have emerged as an important class of bioactive natural products (Shanmugam *et al*., 2000).Thus, medical and pharmaceutical industries are interested since marine plants have proven to be rich sources of structurally diverse bioactive compounds with valuable pharmaceutical and biomedical potential (Madhusudan *et al*., 2011). Sulfated poly-saccharides isolated from seaweeds were investigated to possess immune stimulating (Yeh *et al*. 2006), antitumoral (Dias *et al*., 2005), antivirus (Paskaleva *et al*., 2008); immunomodulatory (Chen *et al*. 2011) and anti-inflammatory (Kang *et al*. 2007) properties. Hepatoprotective (Sathivel *et al* 2003, Manoharan *et al*. 2008, Meena *et al*. 2008) and nephroprotective (Ramarajan *et al*., 2012) effect of some marine algae have been also reported.

The neem plant and its derived products such as Nimbecidine have shown a variety of insecticidal properties on a broad range of insect species (Isman, 2006, Dewer *et al*. 2012). It was reported that, neem extracts have a wide range of effects against insect pests including repellence, antifeedancy, oviposition deterrence, toxicity, sterility and growth regulatory activity (Nathan,1998, Mulla and Su, 1999). These activities are frequently attributed to the azadirachtin, Nimbecidine and other constituents of the plant or its products (Mordue and Blackwell, 1993).

Though, neem-based products are considered to be relatively safe towards non-target biota some authors have reported the adverse effects of neem extract on fish (Saravanan *et al*. 2011, Mutawie &Hegazi, 2011) and rats (Katsayal *et al*. 2008).

The present study was carried out in an attempt to investigate the possible protective effect of *Sargassum crassifolia* against Nimbecidine-induced histopathological and ultrastructure alterations in liver and kidney of rats.

2. Material and Methods

Preparation of water extract of *Sargassum crassifolia:*

The marine brown algal species *S. crassifolia* was collected from the red sea Jeddah coast. The

collected samples were washed and freed from epiphytes, sand particles, pebbles and shells and brought to the laboratory in plastic bags. The samples were then, thoroughly washed with fresh water. Then they were shade dried, powdered into fine particles and subjected to extraction.

Aqueous extract of *S.crassifolia* was prepared based on the method of Fujiki *et al*. (1992). Ten g of the milled fronds was added to 300 ml of deionized water, and the suspension was boiled for 3 hrs. The suspension was filtered through a nylon mesh, and the filtrated extract was lyophilized under reduced pressure. The hot-water extract was dissolved in distilled water and prepared to obtain final concentrations 10% & 25% S. *crassifolia* extracts

Toxicity test of Nimbecidine:

The LC_{50} of Nimbecidine was determined during 48 hrs using 100 rats divided into 10 groups administrated with a series of 10 different concentrations of Nimbecidine. The LC_{50} was found to be 5000 mg / kg b.wt.

Animals and Treatment

Healthy male Wistar rats, weighing 120-150 g were housed in polypropylene cages, maintained in a controlled environment under standard conditions of temperature and humidity with alternating 12 h light /dark cycle. The animals were maintained on standard chow diet and water *ad labitum*. After 15 days of acclimatization period they were randomly assigned into four groups of 10 rats each. Group I served as a vehicle control which received normal diet and water. Animals in group II were given a daily dose (1/10 LD_{50}) of Nimbecidine. Animals in group III received an aqueous extract of 10% *S. crassifolia* as the sole drinking fluid for 4 weeks, simultaneously with a daily dose of Nimbecidine (1/10 LD_{50}). Rats in group IV received an aqueous extract of 25% *S. crassifolia* as the sole drinking fluid, simultaneously with the daily dose of Nimbecidine (1/10 LD_{50}). After four weeks of treatment, animals of control and treated groups were sacrificed by cervical decapitation and dissected and samples of liver and kidney were removed for histological and ultrastructural investigations.

Histological & Electron microscopic Studies:

For histological study, paraffin sections 5µm in thickness were prepared and stained by haematoxylin and eosin.

For electron microscopic studies, 1mm x 1mm parts of the target tissues were fixed in 2.5% glutaraldehyde buffered with cacodylate for 2 hrs then postfixed in 2% osmium tetraoxide for 2hrs at 4C°. Specimens were rinsed twice in cacodylate buffer for 10 min before dehydration in increasing concentrations of ethanol and then were embedded in Epon 8124. Ultrathin sections were prepared and double stained with uranyl acetate and lead citrate, then were examined and photographed using transmission electron microscopy.

2. Results:

1-Histopathological Results:

Light microscopic examination of the liver of control rats showed a compact structure. It consisted of several hepatic lobules; each contained a thin walled central vein surrounded by hepatic radiating cords. The hepatic cords are formed of hepatocytes arranged in one or two rows of cells. The hepatic cords are separated by blood sinusoids which are lined by endothelial cells and Kupffer cells (Fig.1).

Daily administration of Nimbecidine dose (1/10 LD_{50}) for 4 weeks induced marked alterations in hepatocytes involving hepatic vacuolization, fragmented chromatin in nuclei and lymphocytes infiltration (Fig.2). In another side congestion in blood vessels, high dilatation of sinusoids and an increase of Kupffer cells were noticed (Fig.3).

However, rats treated with *S. crassifolia* extract (10%) combined with (1/10LD_{50}) Nimbecidine for four weeks showed mild to moderate enlargement in the sinusoids and congestion of blood vessels (Fig.4). While, higher concentration of alga (25%) combined with Nimbecidine for four weeks brought back the cellular arrangement of the hepatic strands around the central vein and reduced necrosis in hepatocytes. Also, it helped to bring the blood vessels to normal condition (Fig.5).

Microscopic examination of kidney sections of the control group showed normal histological structure of renal tubules and glomeruli in the cortical and medullary portions (Fig. 6).

Histological examination of kidney sections of rats treated with Nimbecidine for 4 weeks revealed damage in glomerulus, dilatation in Bowman's capsule and marked tubular cell degeneration in the form of cloudy swelling and hydropic degeneration (Fig. 7).

Concerning the kidney sections of rats treated with *S. crassifolia* extract (10%) and 1/10 LD_{50}Nimbecidine for four weeks, showed some protective effects in comparison with that of group which treated with Nimbecidine only (Fig.8). However, kidney sections of rats treated with higher concentration of alga (25%) plus Nimbecidine for four weeks revealed improvement of the degenerative changes of the tubular epithelial cells as well as glomerulus and Bowman's capsule (Fig. 9).

2- Ultrastructural Results:

Electron micrographs of liver from control animals revealed the normal ultrastructure of hepatocytes. They are characterized by centrally located round nucleus surrounded by slender stacked

rough endoplasmic reticulum (rER) cisternae. Numerous mitochondria were observed throughout the cytoplasm and in close association with the rER. Smooth endoplasmic reticulum (sER), lysosomes, lipid droplets, ribosomes and glycogen particles were also observed in the hepatocytic cytoplasm (Fig. 10).

After 4 weeks of1/10 LD50 Nimbecidine administration, severe damage on nucleus and cytoplasmic organelle of the hepatic cells were observed. Many nuclei appeared shrunken with dilated nuclear envelope. In addition, dense chromatin masses scattered throughout nucleoplasm and marginated along the nuclear envelope. The cytoplasm appeared vacuolated in most hepatocytes and devoid almost of its components. Most mitochondrial profiles exhibited obvious structural changes, while rER cisternae were decreased and fragmented. Throughout the cytoplasm, a random dispersion of free ribosomes and glycogen particles could be also noticed (Fig.11).

However, rats treated with Nimbecidine at the same dose level and *S. crassifolia* extract (25%) for 4 weeks showed marked improvement in the hepatocytic ultrastructure. The electron micrographs of this group demonstrate that alga decreased the irregularities of nuclear envelope and the nuclei appeared almost normal. Also, the rER cisternae and the mitochondria appeared organized and were evenly distributed in the cytoplasm. Primary lysosomes as well as sER could be observed in the cytoplasm. (Fig.12).

Electron microscopic observations of kidney tissue from control rats revealed that the proximal renal tubular cells are lined along the luminal border with long microvilli, collectively formed a brush border (Fig.13). The nuclei are relatively large, spherical with one or more nucleoli. The heterochromatin dispersed all over the euchromatinor formed a thin layer along the nuclear envelope. Numerous tubular and circular mitochondria are scattered throughout the cytoplasm and surrounding the nuclei. Prominent lysosomes, pinocytotic vesicles, rough endoplasmic reticulum rER and ribosomes can be observed in the cytoplasm (Fig.14). In addition, the proximal tubular cells are supported and surrounded with a distinctive basement membrane (Figs.13&14).

Severe renal ultrastructural alterations were evident in kidney rats that received daily Nimbecidine dose (1/10 LD50) for 4 weeks. The proximal tubular cells exhibited cytoplasmic vacuolization, membranous disruption and destruction of cytoplasmic organelle (Fig.15). The brush border was distorted with loss and irregular microvilli. In addition, most nuclei appeared irregularly- shaped with loss chromatin materials while others showed severe pyknosis (Fig.16). The mitochondria lost their natural shape and inner composition and appeared completely damaged (Figs.15&16). Other organelles could not be easily recognized.

Animals treated with 1/10 LD50 Nimbecidine and *S.crassifolia* extract (25%) for 4weeks showed that the kidney tissue has nearly maintained its natural appearance (Fig.17). Most of the proximal tubular cells appeared in their normal size and structure with existence of slight cytoplasmic vacuolization.

It is obvious that the alga helped in the disappearance of acute renal changes which appeared after treatment with Nimbecidine only. It reduced the irregularities of the microvilli and the nuclear envelope. Also, the mitochondria, lysosomes and the other organelle had approximately recovered.

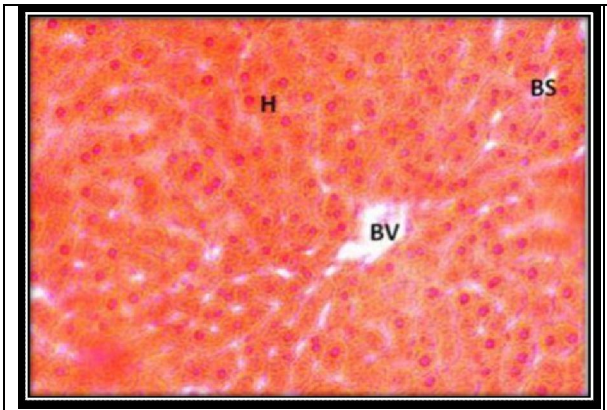

Fig.(1): Light micrograph of liver section from control rats showing the radiating hepatic cords, blood vessel (BV), blood sinusoids (BS) and hepatocytes (H). H&E. x 200.

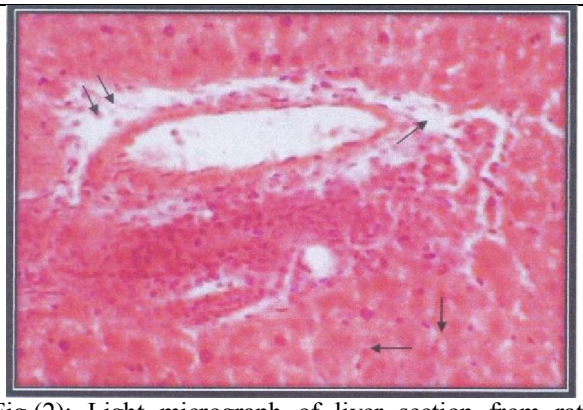

Fig.(2): Light micrograph of liver section from rats treated with Nimbecidine for 4 weeks showing hepatic vacuolization (arrows), degenerated preportal area (double arrows) and leucocytes infiltration. H&E. x 400.

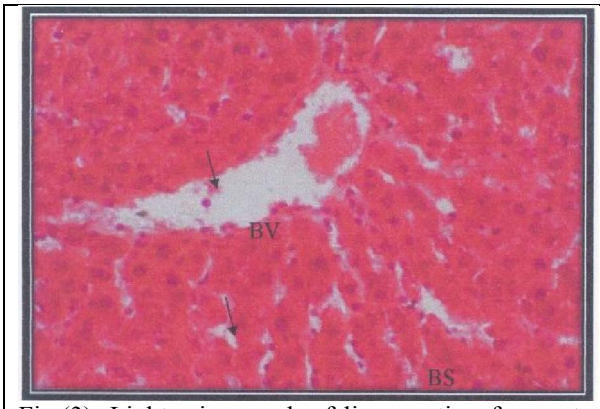

Fig.(3): Light micrograph of liver section from rats treated with Nimbecidine for 4 weeks. Note the congested blood vessel (BV), dilated blood sinusoids (BS) and an increase in number of Kupffer cells (arrows). H&E. x400.

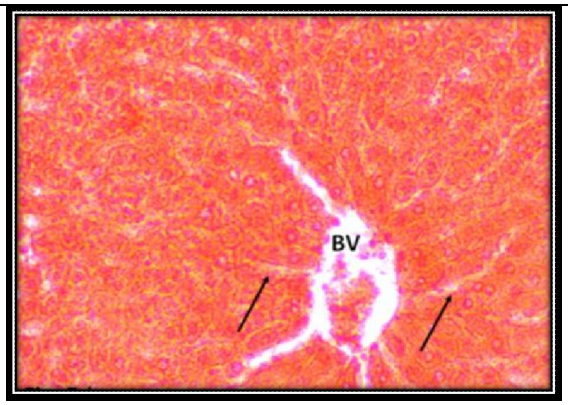

Fig.(4): Light micrograph of liver section from rats treated with 10% *S.crassifolia* and Nimbecidine for 4 weeks. Note mild enlargement in sinusoids (arrows) and congestion of blood vessel (BV). H&E. x 200.

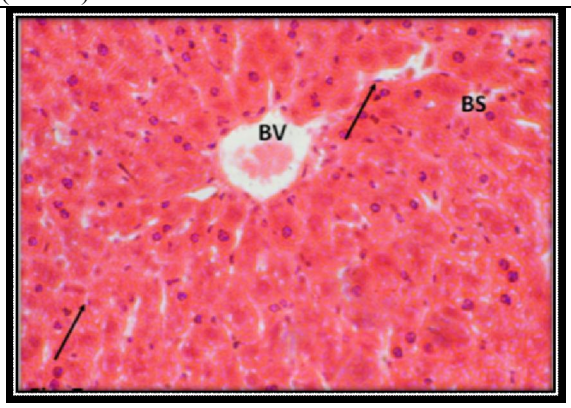

Fig.(5): Light micrograph of liver section from rats treated with 25 % *S.crassifolia* and Nimbecidine for 4 weeks showing the normal cellular arrangement of the hepatic strands, Blood vessel (BV), blood sinusoids (BS) and Kupffer cells (arrows) H&E. x200.

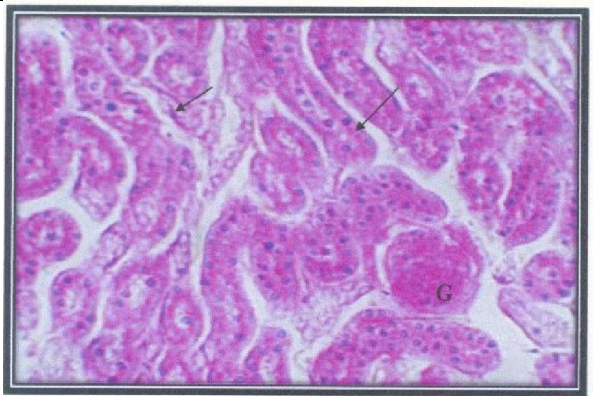

Fig.(6): Light micrograph of kidney section from control rats showing the renal tubules (arrows) and glomerului (G). H&E. x 400.

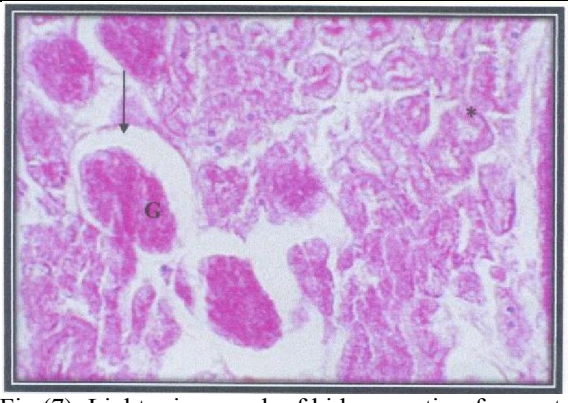

Fig.(7): Light micrograph of kidney section from rats treated with Nimbecidine for 4 weeks. Note damage in the glomerului (G), dilatation in Bowman's capsule (arrow), and degeneration of renal tubules (star).). H&E. x 400.

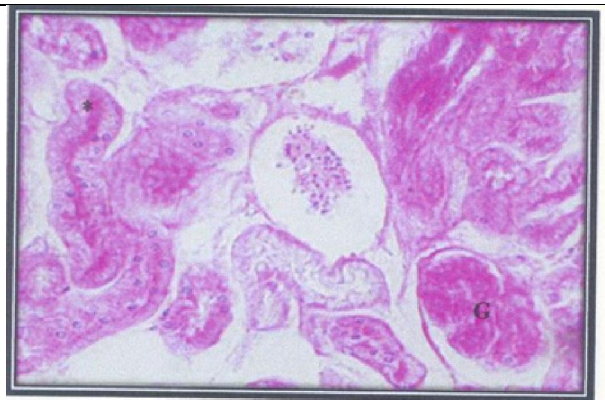

Fig.(8): Light micrograph of kidney section from rats treated with 10% *S.crassifolia* and Nimbecidine for 4 weeks showing some protective effects in renal tubules (star) and glomerului (G). H&E. x 400.

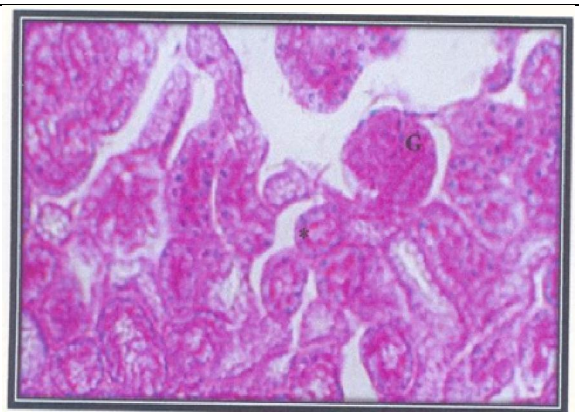

Fig.(9): Light micrograph of kidney section from rats treated with 25 % *S.crassifolia* and Nimbecidine for 4 weeks. Note the improvement of the tubular epithelial cells (star) and glomerului (G). H&E. X 400.

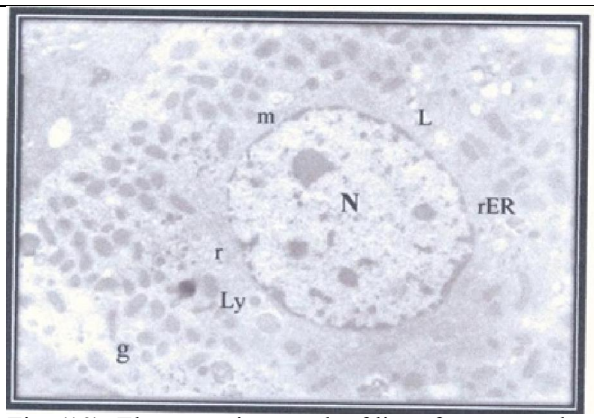

Fig. (10): Electron micrograph of liver from control rat showing the hepatocytes with central nucleus (N), mitochondria (m), rough endoplasmic reticulum (rER), lysosomes (Ly), lipid droplets (L), glycogen particles (g) and ribosomes (r). X 5000.

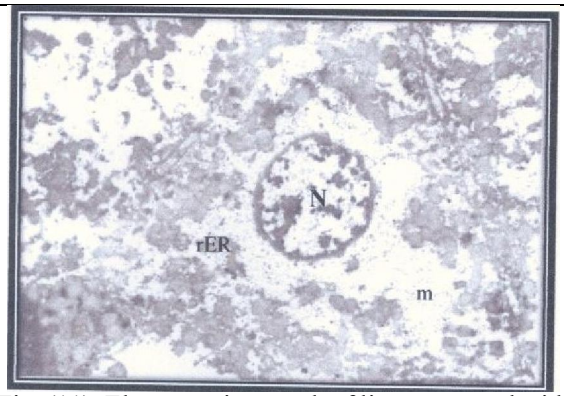

Fig. (11): Electron micrograph of liver at treated with Nimbecidine for 4 weeks. Note vacuolization of cytoplasmic hepatocytes, absence of rER cisternae, altered mitochondria (m) and necrotic nucleus (N). X 3000

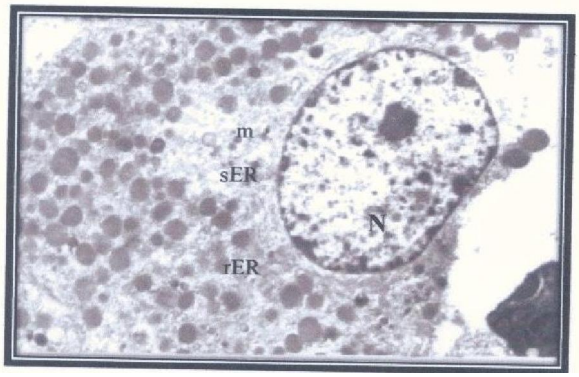

Fig. (12): Electron micrograph of liver rat treated with Nimbecidine and 25 % *S.crassifolia* for 4 weeks.Note the improvement of the nucleus and the cytoplasm of hepatic cells. N, nucleus; m, mitochondria; rER, rough endoplasmic reticulum; sER, smooth endoplasmic reticulum. X 5000

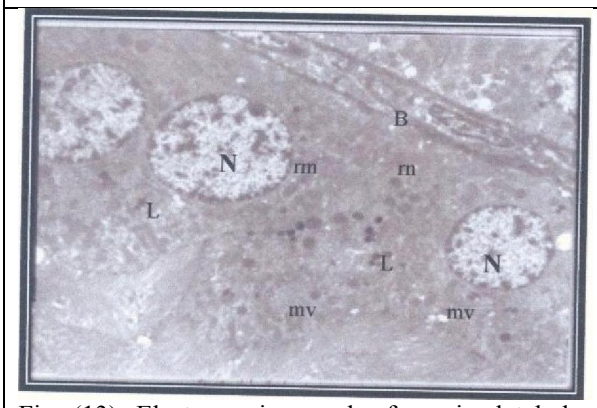

Fig. (13): Electron micrograph of proximal tubular epithelium of kidney from control rat. The luminal border is lined with microvilli (mv) and the tubular cells surrounded with basement membrane (B). L, lysosomes; m, mitochondria; N, nucleus. X 3000

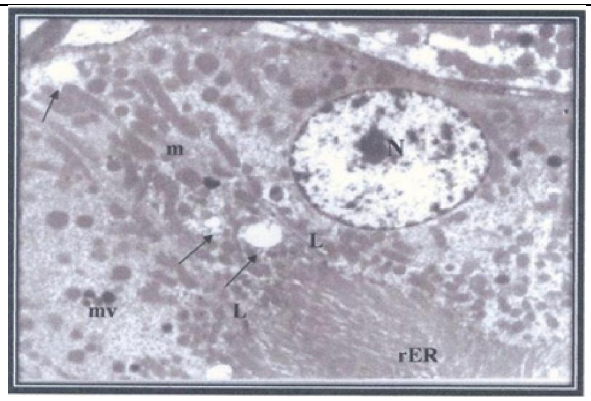

Fig. (14): Higher magnification of one proximal tubular cell of kidney from control rat showing the circular nucleus (N) and the distribution of mitochondria (m) throughout the cytoplasm. Rough endoplasmic reticulum, rER; L, lysosomes; pinocytotic vesicles (arrows). X 4000.

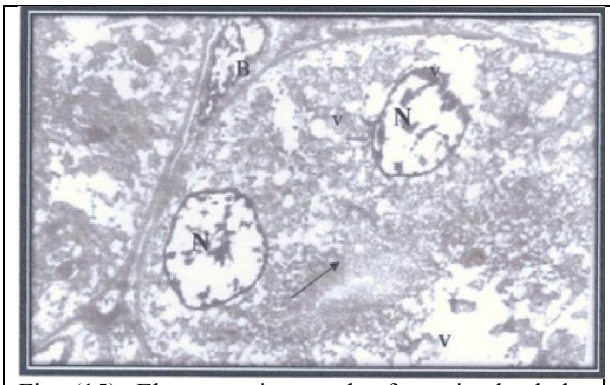

Fig. (15): Electron micrograph of proximal tubular cells of kidney rat after treated with Nimbecidine for 4 weeks. Note cytoplasmic vacuolization (V), destruction of the cytoplasmic organelle, irregular nuclei (N) and distorted brush border (arrow). X 3000

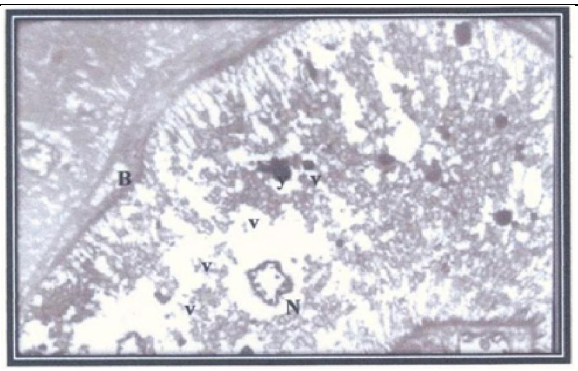

Fig. (16): Electron micrograph of proximal tubular cells of kidney rat after treated with Nimbecidine for 4 weeks. Note highly vacuolized cytoplasm (V), severe damaged organelle, pyknotic nucleus (N), distorted brush border (arrow). X 2000

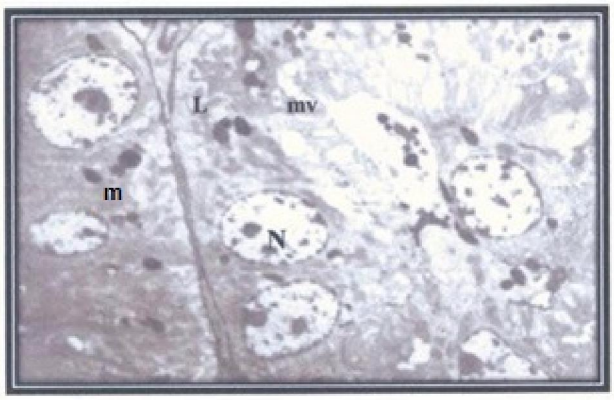

Fig. (17): Electron micrograph of proximal tubular cells of kidney rat after treated with Nimbecidine and 25 % *S.crassifolia* for 4 weeks. The tubular cells appeared nearly with their normal ultrastructure appearance. L, lysosomes; m, mitochondria; mv, microvilli; N, nucleus. X2500

4. Discussion:

The present study was designed to evaluate the possible protective effects of brown alga *Sargassum crassifolia* on liver and kidney tissue of rats after induced injury with the phyto pesticide Nimbecidine.

Previous studies indicated that treatment with Nimbecidine induced pathological, and biochemical disturbances in experimental animals as muscle damage of fresh water fish, *Oreochromis niloticus,* (Mutawie and Hegazi, 2011), histolo-gical changes in the seminal vesicle of adult male *Odontopus varicornis* (Hemiptera: Pyrrhocoridae) (Kumar, *et al*. 2008) and histology-cal changes in the alimentary canal (foregut, midgut and hindgut) of *Sphaerodema rusticum* (Heteroptera: Belastomidae) (Elanchezh-iyan, 2007).

During this study the histopathological examination of liver sections of rats treated with Nimbecidine revealed marked hepatotoxicity in the form of vacuolar degeneration of most hepatocytes, pyknotic nuclei, dilatation and congestion of blood vessels, lymphocyte infiltration and an increase in Kupffer cells.

Similar alterations in liver hepatocytes and nuclei have been observed as a consequence of the insecticides administration in rats by Profenophos (Morsy, 2003) and by Deltamethrin **(**Chargui, *et al.*, 2012). Meanwhile, other studies confirmed that pesticides malathion and chlorpyrifos caused hepatotoxicity (Kalender *et al*., 2010 and Mansour and Mossa, 2010).

Moreover, electron microscopic observations in the present study revealed prominent degenerative changes in liver tissue after Nimbecidine treatment including shrunken nuclei, dilated nuclear envelope and marginated chromatin along the nuclear envelope. In addition, the cytoplasm appeared vacuolated and devoid almost of its components.

Similar ultrastructural alterations were observed in liver rats exposed to the pesticide

carbaryl (TosLuty *et al*.,2001), in liver of mosquito fish exposed to the molluscide-bayluscide (Shoman, 2001) and in liver of fresh water Catfish exposed to the pesticide endosulfan (Rizwan and Chamd,2011).

Our results showed that the renal tissue is adversely affected by Nimbecidine. The histopathological changes were damage in the glomeruli with dilatation in Bowman's capsules and marked tubular cell degeneration.

In previous study the degeneration and necrosis of the renal tubules have also been observed by Fukuoka *et al*. (1987& 1988) and Alden *et al*. (1989) in rats given organo-phosphorous compounds. Recently, similar results were reported by Afshar *et al*. (2008); Al-Attar (2010) Mossalam *et al*. (2011) and Chargui *et al*. (2012) in kidney rat treated with pesticides. Mansour and Mossa (2010) stated that kidney is a target organ of the experimental animals attacked by organophosphorous compounds.

The electron micrographs of the current results showed that the major ultrastructural abnormalites occurred in kidney tissue due to Nimbecidine treatment were cytoplasmic vacuolization and destruction of cytoplasmic organelles of tubular cells. The brush border was distorted with loss and irregular microvilli. Most nuclei appeared irregular in shape with loose chromatin materials and the mitochondria lost their normal shape and appeared damaged.

Similar toxic effects on kidney ultrastructure in albino rats have been reported by Salem (2011), as a result of using pesticide mancozeb. Poovala *et al*. (1999) and Caglar *et al*. (2003) suggested that organophosphate- induced oxidative stress which may play a role in changing configuration of cell membrane and cause injuring and alterations in kidney.

The results of the present study demonstrated that the intensity of the degenerative and necrotic changes of hepatocytes was improved in rats treated with Nimbecidine and alga when compared with that of Nimbecidine – intoxicated rats. This emphasizes that treatment with alga *S.crassifolia* considerably prevented the alterations in liver structural integrity triggered by Nimbecidine and restored the induced histopathological abnormalities.

Madkour *et al*. (2012) suggested that marine alga *S. dentif* extract possess a protective activity against CCl4-induced liver damage in rats. They concluded that the hepatoprotective effects of *S. dentif* may be due to improving the structural integrity of the hepatocyte as a result of their antioxidant activity, which enhance ability to scavenger free radicals and inhibit lipid peroxidation, which are capable of hepatocellular injury.

Furthermore, an improvement of the histological picture of kidney toward the normal was clearly observed in this study after administration of alga *S. crassifolia* with Nimbecidine, which indicates the antioxidant protective effect of the alga and its scavenger effect. Similar nephroprotective effects of alga *C. sinuosa* was reported by Ramarjan *et al*. (2012) against carbon tetrachloride induced kidney injury in rats. Lekameera *et al*. (2008) and Vijayabaskar *et al*. (2012) have concluded that the significant free radical scavenging activity of alga *C. sinuosa* could be a potential source for natural antioxidant.

From the above discussed results, it could be concluded that brown alga *S.crassifolia* has therapeutic and preventive efficiencies against the Nimbecidine induced hepatotoxicity and nephrotoxicity.

Acknowledgements:

We thank the Institute of Research and Consulting, Umm Al-Qura University and our appreciation is expressed to SABIC for their financial support.

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11/12/2013