The histological change resulting from the oxidative stress and the role of *Portunus armatus* extract as anti-inflammatory treatments in rats.

Noor Adnan Azawy *

Assistant lecturer\ veterinary medicine\ department of microbiology\ Tikrit University
Gmail: noor_az@tu.edu.iq

ABSTRACT

The ***Portunus armatus*** is a crustacean that has high economic value, and a wide geographical distribution and medicinal using and considers being sea food in many counters as Iraq. This study aimed to know the treatment effect of ***portunus armatus*** shell extract against oxidative stress and the role in histopathological treatment.

The study included the 15 experimental rats aged 2-3 months distributed to three groups 5 animal of each, the first was given distilled water for four weeks as a control group, while the second group was given hydrogen peroxide at concentrations 1% for two weeks orally using gavage, the third group was given H_2O_2 for two weeks 1,% and then treated with ***Portunus armatus*** extract for tow week. At the end the period of the experiment the animals were scarified. The animals were dismembered, and samples from the liver and spleen were transported to the lab for analysis and tissue segmentation. For the histopathology study, liver and spleen tissues were taken, where it's kept in formalin until the segmentation.

There was a significant histological change of liver and spleen in animal's exposure to oxidative stress by causing much destruction of liver and spleen tissue cells, these effect decrease with the using of *Portunus armatus* extract significantly.

Keywords: oxidative stress, Portunus armatus, histological changes, liver, spleen

Introduction:

The ***Portunus armatus*** is a crustacean that has high economic value, and a wide geographical distribution and medicinal using and considers being sea food in many counters as Iraq**.** Nephropid crabs are deep-sea species that are typically found at depths of 150 to more than 1893 feet (Neg, 1999). Nephropid crabs are typically underside with a penchant for soft substrates. The family Nephropidae now has 57 species that are classified into 14 genera. The crabs of this family are deep-sea forms, and living in their own burrows is a biological behavior in some species (Jones and Morgan, 2002; Nicholas and Chaoshu, 2016). Previously, Jenkins, 1972 split the genus Metanephrops into four morphological groupings, including "thomsoni , binghami , arafurensis and japonicas" . However, with molecular Analysis approach (,Lai et al., 2010) refuted monophyl of the arafurensis and thomsoni groups. Among the groups, blue swimming carb *Portunus pelagicus* has the highest number of species. Some of the current researches on "Indonesian Crustaceans, reported the presence of first records Species, especially hippoid crabs, such as *Albunea Symmysta"* [6]

Living things produce reactive oxygen species (ROS) as a result of regular cellular metabolism and environmental influences like air pollution or smoking cigarettes (Masella et al., 2015). ROS are very reactive chemicals that can harm cellular components such proteins, lipids, carbohydrates, and nucleic acids as well as

change how they operate. When the ratio of antioxidants to oxidants shifts in favor of oxidants, it is referred as "oxidative stress." Redox state regulation is essential for the viability, activation, proliferation, and organ function of cells. Aerobic organisms have integrated antioxidant systems that often work well to counteract the negative effects of ROS. These systems include both enzymatic and non-enzymatic antioxidants. (Mezzetti et al., 2009). However, pathological circumstances can overtax the antioxidant systems. Numerous pathological situations and diseases, such as "cancer, neurological disorders, atherosclerosis, and hypertension", are influenced by oxidative stress. (Scandalios et al., 2014)

This study aimed to studying the histological changes associated with oxidative stress and comparing them after treatment with ***Portunus armatus*** extract.

Material and methods:

Experimental design:

The total number of animals (experimental rats) is 15 , 5 for each group, and were distributed into

1. Five animals as a control group were given water and normal feed.
2. Five animals were given hydrogen peroxide (oxidized) for two weeks.
3. Five animals were given hydrogen peroxide for two weeks and were treated with Portunus ***armatus*** extract foe additionally two weeks.

After the end of experment, the animal was scarified, , and the organs (liver, spleen) were taken for analysis and tissue cutting.

The Method of *Portunus armatus* shel extracting :

Extracting the shell:

The shell is extracted by taking it from the animal, after extracting the organs, cutting the legs, cleaning the shell from the inner well, and then drying it at room temperature, for 3 week. Then grinding it into a fine powder, 25g of the powder was taken and diluted with distilled water at a dilution of 25%. It was ready for using.

Histological examinations

Preparation of samples for histological study:

A. Sampling and Fixation:

The organs of the study were separate by bisected to 3 small specimens (10×5×5 mm) representing the all portion were taken from these organs (liver and spleen). the samples Fixation made by using (10%) buffer formalin saline "Neutral buffered formalin-10 ml, practical grade 37 to 40% formaldehyde solution, distill water 90 ml, sodium phosphate monobasic 0.4 gm, and sodium phosphate dibasic 0.65 gm" for 24-48 Hours. "Dehydration, Clearing, Embedding, Blocking, Sectioning, slide mounting and rehydration were done until obtain the histological section".

Statistical analysis

The study data results were statistically analyzed using the (SPSS) statistical program version (23) by calculating the mean and standard deviation, and using the T-test to determine the difference of the groups at the level of probeability (0.05), (0.01).

Group 1 (control group) liver and spleen

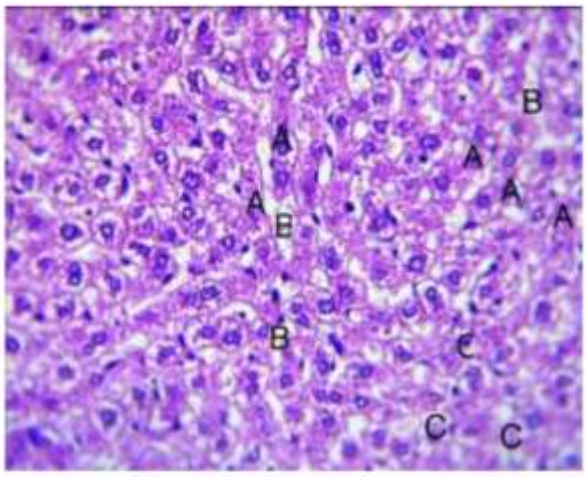


Fig 1

Columns of liver cells (A) White pulp cytoplasm, blood sinusoids (B) kuppfer cells (C)(H&E X40)

The liver cell columns were seen these cells were commonly polygonal shape with spherical nuclei, the cytoplasm of certain cells were containing pale cytoplasm, the blood Sin

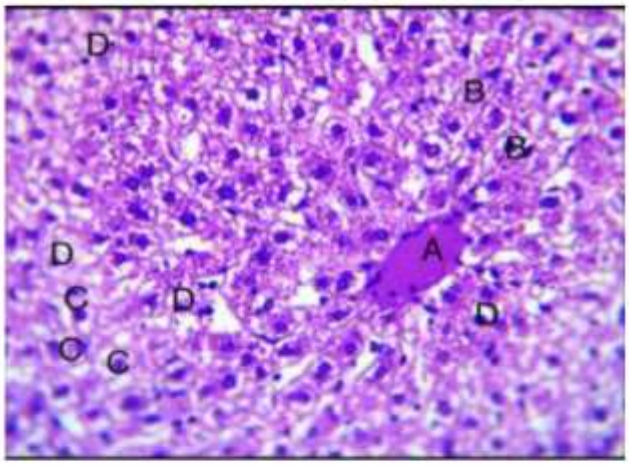


Fig2

Central vein with blood hemolysis (A)

Groups of liver cells (B) Basophilic central nuclei (c) Kupffer cells (D)

(H&E X40)

Sinusoid were presented in network channel, with kuppffer cells inside its lumens.

the central vein have hemolysis blood and surrounded by the groups of liver cells which were crowded to each other and each cell have central basophilic nucleus with presence pale stain of cytoplasm, Kuepfer cells were pre sent in blood Sinusoids.

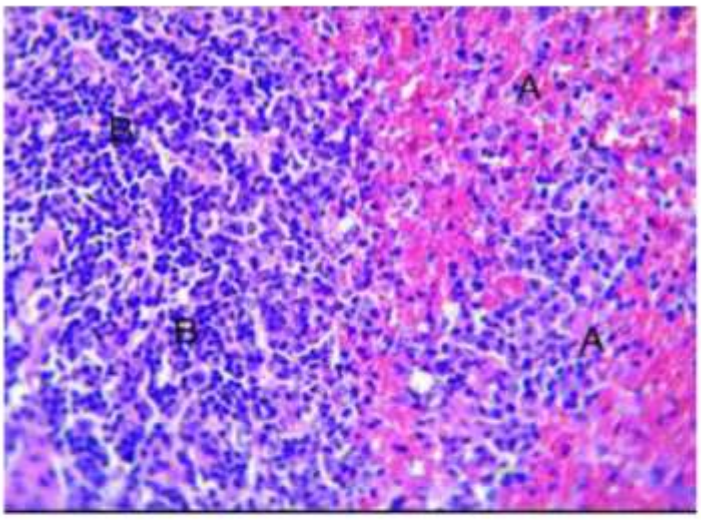


Fig 2

Splenic parenchyma, splenic venous sinuses with blood (A) White pulp with lymphocytic nodular arrangement (B) (H&E X40)

The parenchyma of spleen was containing the splenic venous sinuses or plexus which were engorged with blood and diffuse WBCs, and these are surrounded by lymphocytic nodular aggregation with other WBC, Which form the white pulp and the red pulp was called for splenic venous Sinusoid.

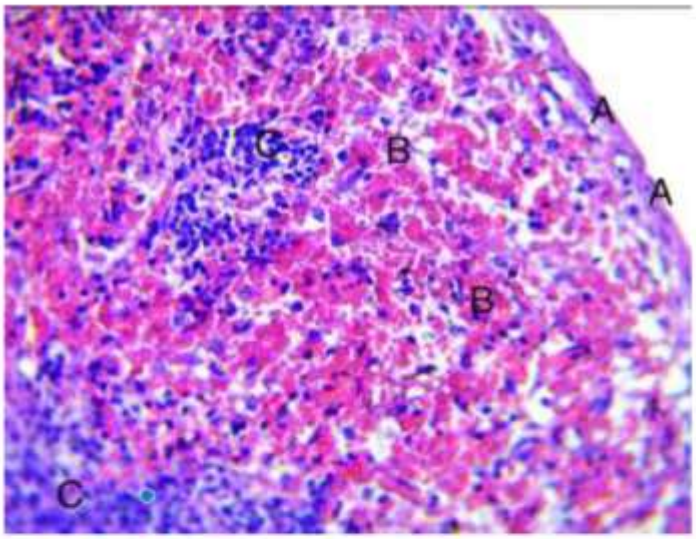
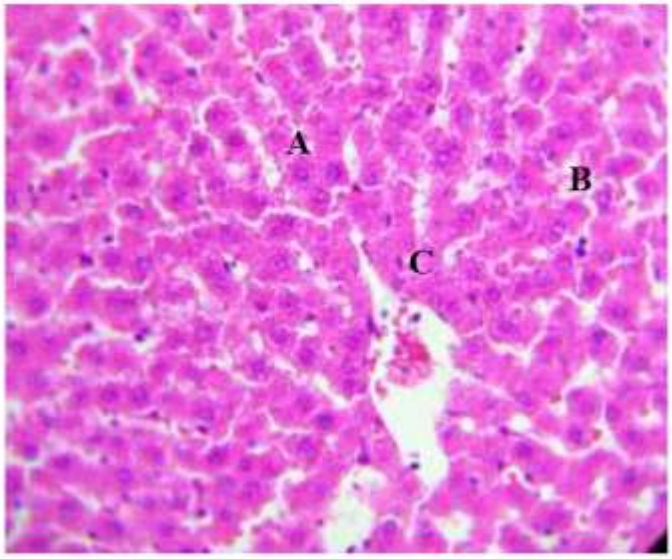


Fig 3

Fibrous capsule of spleen (A) Blood sinuses of red pulp (B) Group of WBCs (C) (H&E X40)

The periphery of spleen was surrounded by fibrous capsule and the parenchyma of spleen was containing many sinuses filled with blood and aggregations with scattered infiltration of the WBCs, lymphocytic aggregations were seen and white pulp in the splenic tissue in different regions of spleen.

Group 2 (oxidative by H_2O_2)



The parenchyma of liver we containing liver lobules (A) , each lobules we formed by liver cells in the form of columns, each liver cells we polyhedral shape with spherical nucleus (B) in the center of cytoplasm and the liver cells were surrounded by blood sinusoid C containing kupffer cells, the blood sinusoid drain the blood in central vein which had blood are surrounded by white blood cells (fg4).

FIG (4) the liver tissue

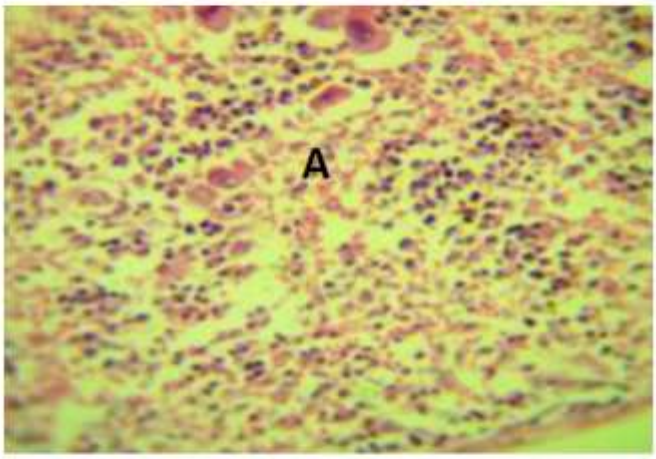


Fig (5) Parenchyma of capsule (A) parenchyma empty of blood, lymphocytes, plasma cells and Purknji cells (B),. (H&E, x40)

Group 3 (the extract treatment group)

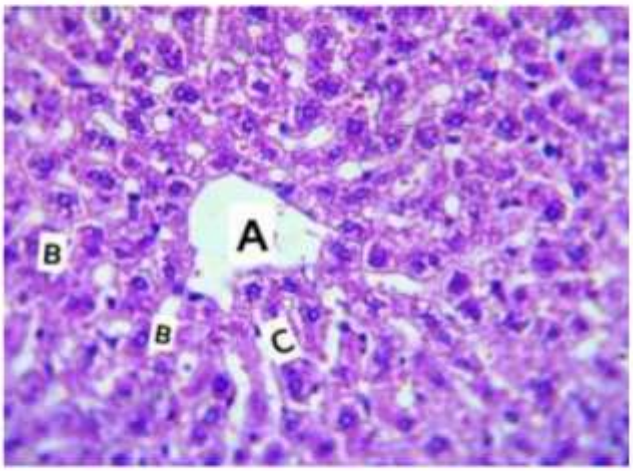


Fig 6 (liver tissue)

Central vein (A) blood sinusoid with kupffer cells (B) liver cell columns (C) with micro vacuoles in its cytoplasm (H & E X 40)

The central vein was present in the liver lobule which appeared continuous from its periphery with blood sinusoid which has the Kuepfer cells, the liver cells were present in radial pattern around the central vein, its cytoplasm had small or microvacuole with spherical basophilic nucleus (fig 6)

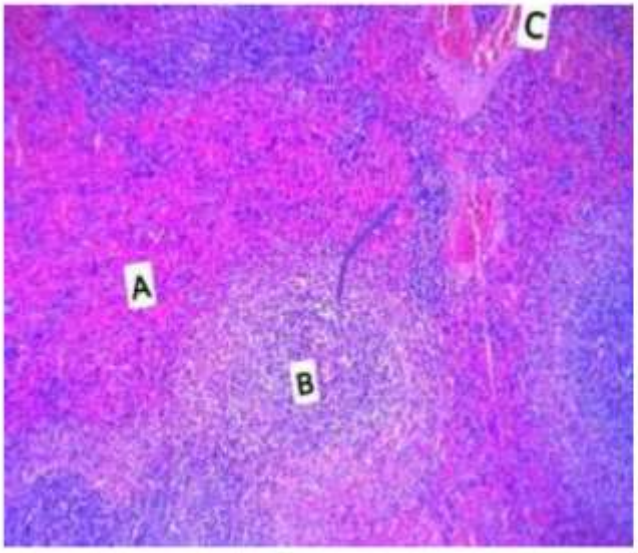


Fig 7 (spleen tissue)

Splenic parenchyma red pulp with splenic venous plexus (A)white pulp with nodular lymphocyte aggregation (B),splenic artery (C)

(H & E X 40)

The splenic parenchyma was arranged into splenic venous plexus (red pulp) which were engorged with blood and diffused WBCs, and nodular aggregation of WBCs other lymphocyte (white pulp) which had germinal center (fig 7)

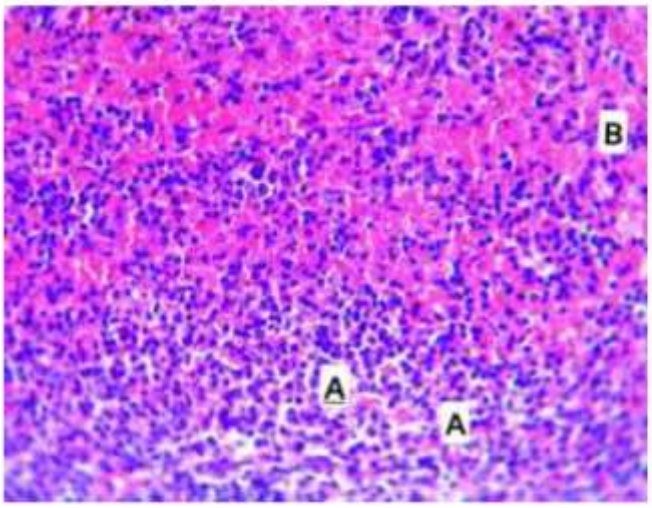


Fig 8

Red pulp with blood congestion (A) lymphocyte aggregation of white pulp (B) (H & E X 40)

The red pulp was engorged with blood and other WBCs; those are surrounded by nodular aggregation of which pulp of lymphocyte (fig 8).

The current study revealed an increase in the systemic inflammation inside the tissue during the histopathological examination of the liver and spleen, and after the oxidative effect by hydrogen peroxide, this is agree with Ozougwu, (2014). Hepatocytes were lysed and necrosed, and the central vein was congested. These findings were consistent with those of Mezzetti et al. (1997), who also observed the same alterations.

The harmful effects of H2O2 and its metabolites on the liver could be the cause of the current histological findings, according to Masella et al. (2015). Oxidative stress causes lipids, proteins, and DNA to be disrupted, causes hepatocytes to necrotize and apoptose, and intensifies the inflammatory response (Scandalios, 2014), and it promotes the Kupffer cells' and circulating inflammatory cells' release of profibrogenic mediators, which leads to the beginning of fibrosis. (Mezzetti et al. ,1997) .

The blue swimming carb shell play important role as anti-oxidant because its contain many material and metallic ions such as potassium and calcium, zinc and protein which act as protective agents of the tissus (Makalani et al., 2217; Kang et al., 2019; Nicholas and Chaoshu ,2017).

Conclusion

The current findings approved that the oxidative stress could cause hepatic and splenic tissue damage, supported by histopathological changes in both organs. The effect of ***Portunus armatus*** extract is clear as anti-oxidant by improvement the tissues texture. Future research should also look into the other extract effect on other organs such as the brain, heart, and kidney.

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