Journal of Bioscience and Applied Research

The possible anti-inflammatory role of the blue green algae, Aphanizomenon flos-aquae on adult male rats

Samir Atea Mohammed1, Hemat Mansour Abdelhafez1, Fatma Ahmed Eid1, Ola Mohammed Abdel-Raouf2, Rasha Mohammed Ibrahim1

1. Department of Zoology, Faculty of Science, Al- Azhar University, Cairo, Egypt.

2. Pharmacology Department, National Organization for Drug Control and Research (NODCR), Cairo, Egypt.

Résumé :

L'Aphanizomenon Flose-aquae (AFA) est une microalgue bleu-vert unicellulaire d'eau douce comme la spiruline, mais la plupart de l'AFA est récoltée à l'état sauvage dans les régions volcaniques menant à des niveaux élevés d'oligo-éléments. Les Aphanizomenon Flos Aquae ont été traditionnellement utilisées depuis plus de 25 ans pour leurs propriétés bénéfiques pour la santé.

Pour étudier le rôle anti-inflammatoire possible de l'Aphanizomenon Flose-aquae des rats ont été injectés avec du carraghénane.

(Le carraghénane (ou carraghénine) est un polysaccharide (galactane) extrait d'algues rouges servant d'agent d'épaississement et de stabilisation dans l'industrie alimentaire. Il porte le code E407 de la classification des additifs alimentaires. Les carraghénanes permettent de former des gels à chaud (jusqu'à 60 °C) et présentent donc un intérêt par rapport aux gélatines animales traditionnelles. Des scientifiques ont été amenés à se poser de sérieuses questions sur la toxicité de cet additif dans l'alimentation. À partir d'études effectuées sur des animaux, il a été démontré des cas d'inflammation intestinale, des ulcérations et des coliques en ajoutant cet additif alimentaire dans l'eau de boisson. Certains praticiens recommandent d'éviter la consommation d'aliments contenant des carraghénanes, spécialement pour les personnes souffrant de problèmes gastro-intestinaux).

Les résultats obtenus dans la présente étude ont montré que l'injection de rats avec du carraghénane a conduit à une diminution significative du nombre de globules rouges (hématies) de comptage, de la concentration d'hémoglobine (Hb) et de la valeur d'hématocrite (HCT) par rapport à ceux du groupe témoin.

En revanche l'activité a été significativement augmenté au niveau des globules blancs (leucocytes) comptant, l'alanine aminotransférase (ALT), l'aspartate aminotransférase (AST) et la phosphatase alcaline (ALP). Cela a été accompagnée par des changements histopathologiques et histochimiques.

Pendant ce temps, le traitement des rats avec AFA pendant cinq et vingt et un jours n'a pas montré d'effet toxique sur le foie. En outre l'activité anti-inflammatoire a présenté une amélioration des paramètres hématologique de la fonction hépatique dans le traitement des rats avec injection d'AFA post-carraghénane. En conclusion, l'utilisation d'Aphanizomenon Flos Aquae comme agent naturel a montré un puissant rôle anti-inflammatoire.

Extrait de la publication

Journal of Bioscience and Applied Research,2016 , Vol.2, No.6, PP:414-425 pISSN: 2356-9174, eISSN: 2356-9182

ci-dessous

Journal of Bioscience and Applied Research

JBSAR

WWW.JBSAR.com

Society of Pathological Biochemistry and Haematology

The possible anti-inflammatory role of *the blue green algae ,Aphanizomenon flos-aquae* on adult male rats

Samir Atea Mohammed[1], Hemat Mansour Abdelhafez[1], Fatma Ahmed Eid[1]
Ola Mohammed Abdel-Raouf[2], Rasha Mohammed Ibrahim[1]

1. Department of Zoology, Faculty of Science, Al- Azhar University, Cairo, Egypt.
2. Pharmacology Department, National Organization for Drug Control and Research (NODCR), Cairo, Egypt.

(Corresponding author email: fatmaeid22@yahoo.com)

Abstract

Aphanizomenon flose-aquae (AFA) is a fresh water unicellular blue green microalgae like *Spirulina*, but most AFA is harvested from the wild in volcanic regions leading to high levels of trace minerals. *Aphanizomenon flos-aquae* have been traditionally used for over 25 years for its health-enhancing properties**.** To investigate the possible anti-inflammatory role of *Aphanizomenon flose-aquae*, rats were injected with carrageenan. Results obtained in the present study showed that injection of rats with carrageenan led to significant decreases in red blood cells (RBCs) count, hemoglobin concentration (Hb) and hematocrit value (Hct) as compared to those of the control group. In contrast, the levels of white blood cells (WBCs) count, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities were significantly increased; this was accompanied by histopathological and histochemical changes. Meanwhile, treatment of rats with AFA for 5 and twenty one days did not exhibit a toxic effect on the liver. Also, treatment of rats with AFA post-carrageenan injection exhibited anti-inflammatory activity and improved hematological parametrs and liver function profile. In conclusion, using *Aphanizomenon flos- aquae* as a natural agent showed a strong anti-inflammatory role**.**

Keywords: *Aphanizomenon flos- aquae***,** Carrageenan, Inflammation and Rats.

1 Introduction

Carrageenan emerged as a fascinating compound has a wide spectrum of interference with the biological systems. Although the safety and toxicity profile of carrageenan is well studied, it was reported that different carrageenan subtypes (iota-, lambda- and kappa-carrageenan) can produce different biological and toxicological effects (Mc-kim, 2014; Weiner, 2014). It is often used for the testing of anti-inflammatory agents (Sini *et al*., 2010). There is a worldwide trend to natural resources, which are culturally acceptable and economically viable. Among the important and effective drugs used to treat chronic diseases are derived from plants and certain species of cyanobacteria (Yeh *et al*., 2006 ; Nahin *et al*., 2009 ; El-Depsi, 2016). Blue-green algae (BGA), also known as cyanobacteria, among the phylum of bacteria that utilize photosynthesis to obtain energy. They are technically classified as bacteria and share properties with them (Schaap *et al*., 2012). They are nutritious natural products rich in essential amino acids, γ-linolenic acid, fibres, B vitamins, calcium, iron, phosphorous, pigments such as β-carotene, xanthophylls, chlorophyll and other bioactive compounds (Regunathan and Wesley, 2006). Singh *et al*. (2005) and El-Depsi (2016) reported that BGA have antiviral, antitumor, antioxidant, anti-inflammatory, anti-allergic, anti-diabetic and antibacterial properties as well as lipid-lowering effects. *Aphanizomenon flos-aquae* (AFA) is a fresh water unicellular blue-green alga that is consumed as a nutrient-dense food source and for its health-enhancing properties (Benedetti *et al*., 2004 ; Scoglio *et al*., 2014). *Aphanizomenon flos-aquae* as a species has both nontoxic and toxic forms. Most sources worldwide are toxic, containing both hepatic and neuroendotoxins. *Aphanizomenon flos-aquae* from Klamath Lake is a non-toxic type of algae of the cyanobacteria phylum (Jensen *et al*., 2001). *Aphanizomenon flos-aquae* have gained popularity in USA, Germany, Canada, Korea, Japan and Austria. It contains 20 antioxidants, 68 minerals, 70 trace elements, all amino acids, B vitamins and important enzymes (Chakdar *et al*., 2012). Also, the most common BGA, *Spirulina platensis* (SP) and *Aphanizomenon flos-aquae* (AFA) were found to have antioxidant (Venkatesan *et al*., 2012), anti-inflammatory and hypolipidemic properties (Tiniakos *et al*., 2010 ; Yang *et al*., 2011). *Aphanizomenon flos-aquae* is an important source of the blue photosynthetic pigment phycocyanin (PC), which has been described as a strong antioxidant and anti-inflammatory agent (Reddy *et al*., 2000 ; Scoglio *et al*., 2009 ; Benedetti *et al*., 2010). The present work studied the antiinflammatory effect of *Aphanizomenon flos-aquae* induced by carrageenan in rats.

2 Materials and Methods

Experimental animals

A total of forty eight mature male albino rats weighing about 120-160 g. each were used in the present study. The animals were kept in the laboratory for two weeks before the experimental work. They were housed in especially designed and cleaned cages, 6 rats per cage and maintained under controlled conditions of temperature, light (12 hours light: 12 hours dark) and good ventilation. They were fed normal diet and water *ad libitum***.**

Experimental protocol

Animals were equally divided into four groups as follows: the first group control (C), the second group injected with carrageenan and left for 21 days (Carr), the third group received *Aphanizomenon flos- aquae* (AFA) extract (94.5 mg/kg body weight /day) for 21 days, the fourth group injected with carrageenan and treated with 94.5 mg/kg body weight AFA extract daily for 21 days (AFA+ Carr). The experimental rats were sacrificed after 5 and 21 days post–treatment. Carrageenan (Carr) solution was prepared as 1% suspension in saline, where each animal was injected by 0.1 ml of carrageenan solution in subplanter tissue of the left hind paw (Ghosh *et al.*, 2000) for induction of experimental inflammation.

AFA extract was prepared by dissolving it in distilled water, then the drug was administered orally by gastric tub for 21days. The dose for rats was calculated according to the Paget's formula on the basis of the human dose (Paget **and Barnes, 1964).** At the end of experiment, peripheral blood was collected for hematological parameters and serum was separated by centrifugation at 3000 rpm for 10 minutes for biochemical analysis. Livers of rats were carefully removed and prepared for various histopathological and histochemical studies.

1-Hematological studies

Red blood cells (RBCs) were counted visually according to the method of Dacie and Lewis (1991). Hemoglobin concentration was determined according to the method of Drabkin and Austin (1932). Hematocrit value was determined according to the method of Rodak (1995) using heparinized capillary tubes. White blood cells (WBCs) were counted visually according to the method of Mitruka and Rawnsley (1977).

2-Biochemical parametrs

The activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed by the kinetic method using available commercial kits (Spinreact, Spain) according to the method described by Young and Friedman (2001). The activities of alkaline phosphatase (ALP) in serum were assayed by the kinetic method using available commercial kit (Spinreact, Spain) according to the International Federation of Clinical Chemistry (IFCC) (Schumann *et al.,* 2002).

3-Histopathological and histochemical techniques

Livers of all groups were washed in saline and fixed in 10% neutral formalin, embedded in paraffin, sectioned at 5 µm and stained with haematoxylin and eosin stain according to the method of Bancroft and Gamble (2002), Mallory's trichrome stain (Pears, 1977), periodic acid Schiff technique (Drury and Wallington, 1980), mercuric bromophenol blue (Mazia et al., 1953) and Congo red stain (Valle, 1986) for light microscopic observations.

3 Results

1-Hematological results

- Red blood cells (RBCs) count

Figure 1 summarizes the data obtained for red blood cells count (RBCs x 10^6 cell/mm^3) in albino rats injected with carrageenan and treated with AFA at different intervals (5 and 21 days post-treatment) in comparison with the control group. Injection of rats with carrageenan induced a significant decrease in RBCs count all over the experimental periods as compared to the control group. At the same time oral administration of albino rats with AFA alone showed non-significant changes (P>0.05) in the mean values of RBCs, while rats treated with AFA post-carrageenan injection resulted in non-significant decreases in RBCs count on the five and twenty one days post-treatment as compared to the control group.

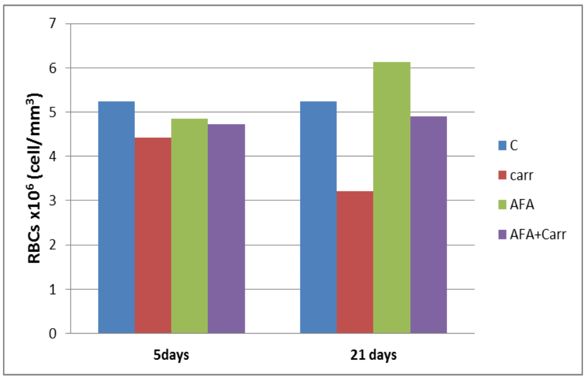


Figure 1. Showing the mean values of red blood cells (RBCs x 10^6 cell/mm^3) in the control and the different experimental groups (Carr, AFA and AFA+ Carr) at different intervals (5 and 21 days post-treatment).

-Estimation of hemoglobin (g / dl) concentration

Figure 2 showing the changes of hemoglobin (Hb) concentration g/dl after treatment of albino rats with carrageenan and/or AFA at different intervals (5 and 21 days post-treatment) in comparison with the control group. The present results showed that injection of rats with carrageenan induced a significant decrease in hemoglobin concentration at 5 and 21 days post-treatment as compared to the control group. Data also showed that drenching AFA to the rats exhibited non-significant changes in Hb concentration (P>0.05). On the other hand, rats treated with AFA post-carrageenan injection presented non-significant changes in hemoglobin concentration at 5 and 21 days post-treatment in comparison with the control group.

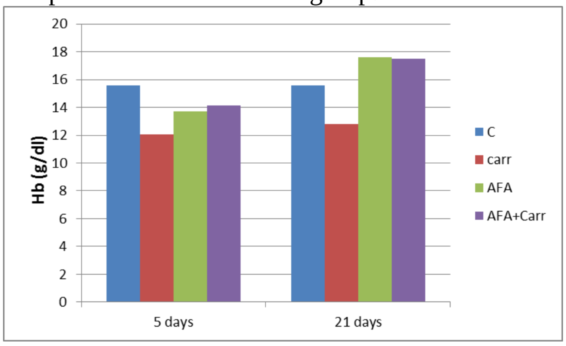


Figure 2- Showing the mean values of hemoglobin (Hb g/dl) concentration in the control and the different experimental groups (Carr, AFA and AFA+ Carr) at different intervals (5 and 21 days post-treatment).

-Measurement of hematocrit (Hct) value

Figure 3 showing the hematocrit percent (Hct %) of male rats treated with carrageenan and/or AFA all over the experimental periods (5 and 21 days post- treatment). Rats injected with carrageenan showed highly significant decrease (P<0.01) in the mean value of blood Hct at 5 days and a very highly significant decrease (P<0.001) after 21 days as compared to the control group**.** On the other hand, rats treated with AFA alone showed non-significant changes (P>0.05) in Hct mean values at 5 and 21 days as compared to the control group. Meanwhile, treatment with AFA post-carrageenan injection exhibited non-significant changes in the mean values of Hct on the 5 and 21 days post-treatment as compared to the control group.

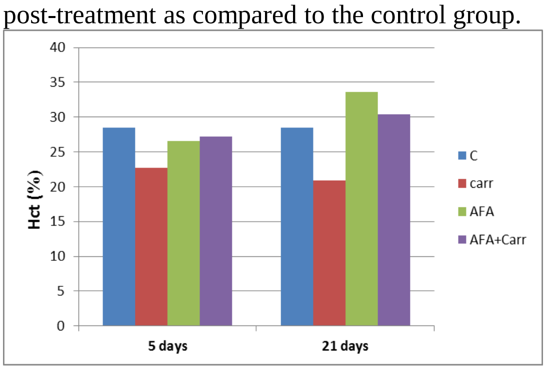


Figure 3- Showing the mean values of hematocrit percent (Hct%) in the control and the different experimental groups (Carr, AFA and AFA+ Carr) at different intervals (5 and 21 days post-treatment).

-White blood cells (WBCs) count

Data of white blood cells (WBCs x10^3 cell/mm^3) after treatment of albino rats with carrageenan and/or AFA at different intervals (5 and 21 days post-treatment) were reported in **figure 4.** It was noticed that injection of rats with carrageenan induced a significant increase (P<0.05) in the mean value of WBCs after 5 days post-treatment as compared to the control group. While, very highly significant increase (P<0.001) was observed in the mean value of WBCs after 21 days pot-treatment.

Also, treatment of rats with AFA alone showed non-significant increase (P>0.05) in mean values of WBCs at 5 and 21 days post-treatment as compared to the control group. On the other hand, treatment of rats with AFA post-carrageenan injection resulted in a non-significant increase in the mean values of WBCs at 5 and 21 days post-treatment as compared to the control group.

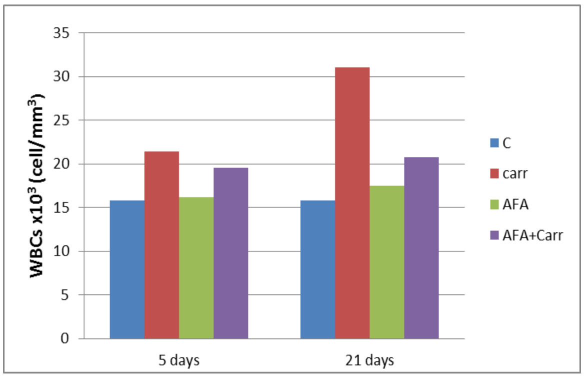


Figure 4 - Showing the mean values of white blood cells (WBCs x 10^3 cell/mm^3) in the control and the different experimental groups (Carr, AFA and AFA+ Carr) at different intervals (5 and 21 days post-treatment).

2-Biochemical parametrs

-Serum alanine aminotransferase activity

Figure 5 showing the levels of alanine aminotransferase (ALT, U/L) in serum of male rats treated with carrageenan and/or AFA at different intervals (5 and 21 days post-treatment) as compared to the control group.

Rats injected with carrageenan exhibited a significant increase (P<0.05) in the mean values of serum ALT level on the five and twenty one days post-treatment in comparison with the control group. The present results also showed that there is a non-significant decrease (P>0.05) in the mean values of serum ALT in rats treated with AFA alone on the 5 and 21 days post-treatment as compared to the control group. On the other hand, treatment of rats with carrageenan and AFA resulted in a non-significant increase in the mean values of serum ALT on 5 and 21 days post-treatment in comparison with that obtained from the control group.

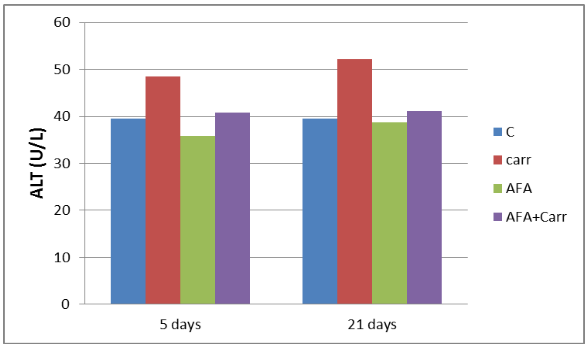


Figure 5 - Showing the mean values of serum alanine aminotransferase (ALT, U/L) in the control and the different experimental groups (Carr, AFA and AFA+ Carr) at different intervals (5 and 21 days post-treatment).

-Serum aspartate aminotransferase activity

Figure 6 showing the levels of AST in serum of male rats injected with carrageenan and/or AFA at different intervals (5 and 21 days post-treatment). Injection of rats with carrageenan recorded a significant increase ($P<0.05$) in the mean values of serum AST after 5 and 21 days post-treatment as compared to the control value. Rats treated with AFA exhibited a non-significant increase ($P>0.05$) in the mean values of serum AST all over the experimental periods as compared to the control group. On the other hand, non-significant increases in the mean values of serum AST were reported in group administrated AFA post-carrageenan injection at 5 and 21 days post-treatment as compared to the control group.

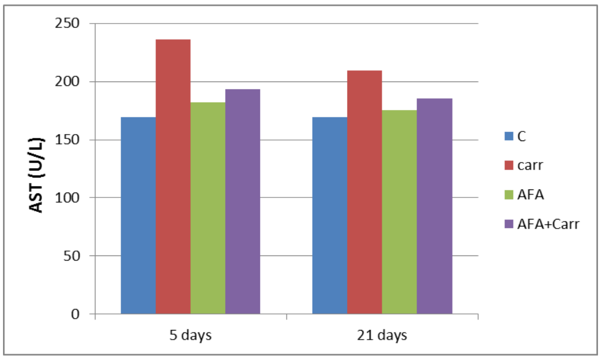


Figure 6 - Showing the mean values of serum aspartate aminotransferase activity (AST, U/L) of the control and the different experimental groups (Carr, AFA and AFA+ Carr) at different intervals (5 and 21 days post-treatment).

Serum alkaline phosphatase activity

Data illustrated in **figure 7** showing ALP activity in serum of male rats treated with carrageenan and/or AFA at different intervals (5 and 21 days post-treatment).

Carrageenan injection to the rats induced a significant increase ($P<0.05$) in the mean values of serum ALP level all over the experimental periods in comparison with the control group. While, non-significant decreases ($P>0.05$) were realized in the mean values of serum ALP level in AFA group at 5 and 21 days post-treatment when compared to the control group. However, non-significant increases were observed in the mean values of serum ALP in group treated with AFA post-carrageenan injection after 5 and 21 days post-treatment as compared to the control group.

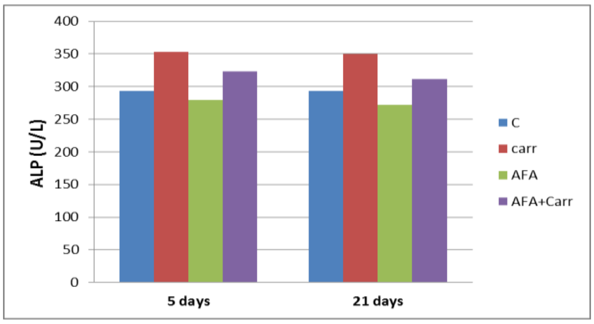


Figure 7- Showing the mean values of serum alkaline phosphatase (ALP, U/L) in the control and the different experimental groups (Carr, AFA and AFA+Carr) at different intervals (5 and 21 days post-treatment).

3-Histopathological and histochemical observation

The histological and histochemical alterations are shown in Figures 8-15. Examination of the liver tissue after five and twenty one days in the carrageenan injected group of male adult albino rats showed aggregated lymphocytes in and around the portal areas with hemorrhagic area. Liver sections also showed highly dilated and congested hepatic portal veins which contained hemolysed blood cells with elongated, distorted and increased proliferation in walls of the bill ducts. Numerous vacuolated hepatocytes and highly increased kupffer cells were also detected. Highly dilated and distorted wall of the central veins with ruptured and delaminated endothelial lining of them were also noticed. Central veins contain hemolysed blood cells inside them.

carrageenan group exhibited a significant increase in PAS positive materials all over the experimental periods. While, rats administrated AFA alone and AFA post-carrageenan injection exhibited non-significant increase in PAS positive materials relative to the control group after five and twenty one days post-treatment. Carrageenan group exhibited a significant increase in the total protein and amyloid content in the liver tissue relative to the control group all over the experimental periodsWhile, treatment with AFA post-carrageenan injection showed somewhat normal deposition of amyloid protein content relative to the control group after five and twenty one days post-treatment.

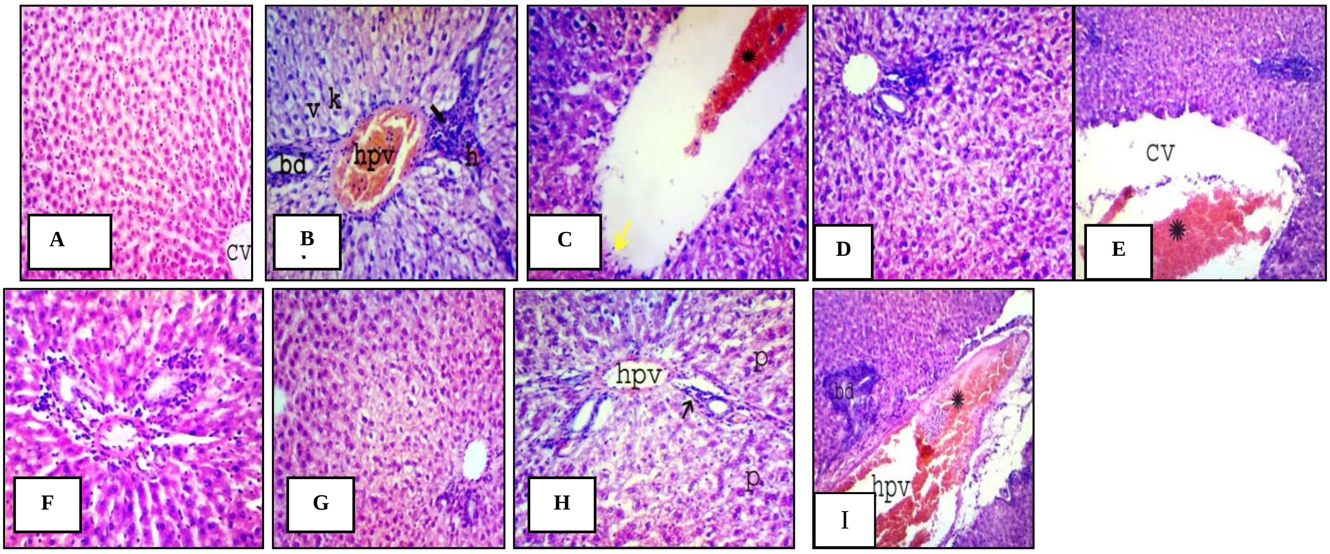


Figure 8- A. A photomicrograph of the liver tissue of the control group showing normal appearance of the central vein (cv), cords of hepatocytes and blood sinusoids. **B**. A photomicrograph of the liver tissue of rats five days post- carrageenan injection showing highly dilated and congested hepatic portal vein (hpv) which contain hemolysed blood cells inside them, elongated and distorted walls of the bill ducts (bd) with increased proliferation in them, aggregated lymphocytes **(→)** in and around the portal areas with hemorrhagic area (h), numerous vacuolated hepatocytes (V) and highly increased Kupffer cells (K). **C.** A photomicrograph of the liver tissue of rats five days post- carrageenan injection showing highly dilated central vein which contains hemolysed blood cells inside it (*) with highly distorted endothelial lining of it **(→)**. **D.** A photomicrograph of the liver tissue of rats injected with carrageenan and examined after twenty one days showing highly congested and dilated hepatic portal veins (hpv) , they contain hemolysed blood cells inside them (*) with highly stratified walls of bill ducts (bd). **E.** A photomicrograph of the liver tissue of rats injected with carrageenan and examined after twenty one days showing highly distorted wall of the central vein (CV) with ruptured and delaminated endothelial lining of it. It contains hemolysed blood cells inside it (*). **F.** A photomicrograph of the liver tissue of rats treated with AFA alone for five days showing normal structure of the portal area. **G.** A photomicrograph of the liver tissue of rats treated with AFA alone for twenty one days showing somewhat normal architecture of the portal area with increased lymphocytes. **H.** A photomicrograph of the liver tissue of rats treated with AFA post-carrageenan injection for five days showing somewhat normal structure of most hepatocytes of the portal area, but some hepatocytes contain pyknotic nuclei (P) with slightly increased lymphocytes **(→)**. **I.** A Photomicrograph of the liver tissue of rats treated with AFA post-carrageenan injection for twenty one days showing well developed portal area,(X300).

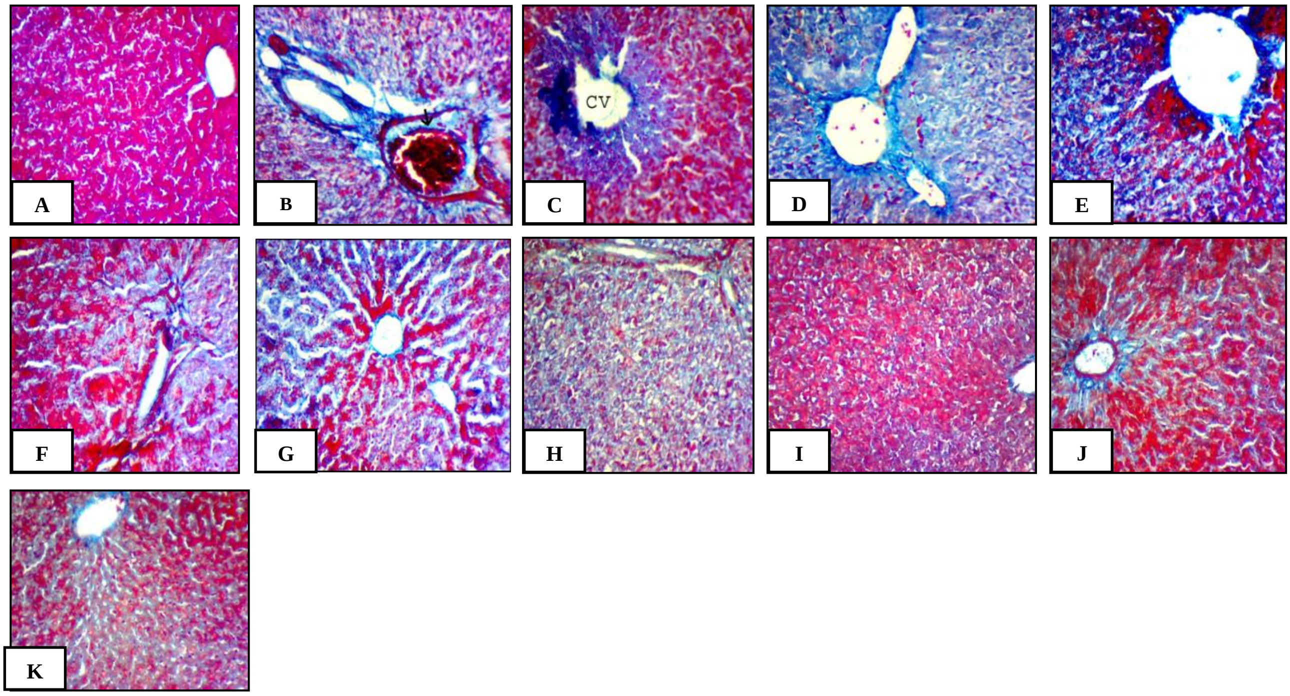


Figure 9- A. A photomicrograph of the liver tissue of the control group showing thin bundles of collagen fibres support walls of the hepatocytes, blood sinusoids and walls of the blood vessels. **B,C.** Photomicrographs of the liver tissue of rats five days post-carrageenan injection showing highly increased collagen bundles around the portal area, inside the hepatic portal veins (→), around the bile ducts and also beside the central vein. **D,E.** Photomicrographs of the liver tissue of rats injected with carrageenan and examined after twenty one days showing highly increased collagen fibres all over the liver tissue especially in walls of the central veins and in the portal area. **F,G.** Photomicrographs of the liver tissue of rats treated with AFA alone for five and twenty one days showing normal distribution of collagen fibres around the hepatocytes, the central vein, portal area and the blood sinusoids. **H,I.** Photomicrographs of the liver tissue of rats treated with AFA post-carrageenan injection for five days showing somewhat normal distribution of collagen fibres around the hepatocytes, central vein, portal area and blood sinusoids. **J,K.** Photomicrographs of the liver tissue of rats treated with AFA post-carrageenan injection for twenty one days showing normal distribution of collagen fibres around the hepatocytes, central vein, portal area and blood sinusoids, (X300).

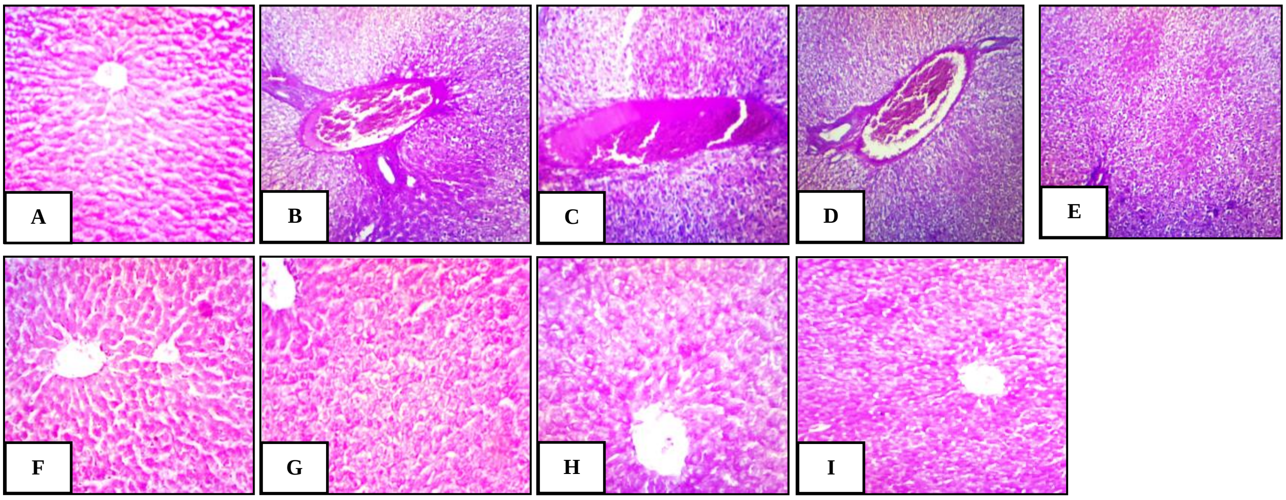


Figure 10- A. A photomicrograph showing normal distribution of PAS +ve materials in the liver tissue of the control group. **B, C.** Photomicrographs showing increased staining affinity of PAS +ve materials in the liver tissue of carrageenan group after five days. **D,E.** Photomicrographs showing increased staining affinity of PAS +ve materials in the liver tissue of carrageenan group after twenty one days. This increase was observed inside and around the highly dilated hepatic portal veins, around walls of the bile ducts and around the hepatocytes. Also, this increase was observed inside and around the highly dilated central vein and in the hemolysed RBCs**.** **F,G.** Photomicrographs showing normal distribution of PAS +ve materials in the liver tissue of AFA group after five (F) and twenty one days(G). **H,I.** Photomicrographs showing somewhat normal distribution of PAS +ve materials in the liver tissue of AFA+ Carr group after five (H) and twenty one days (I),(X300).

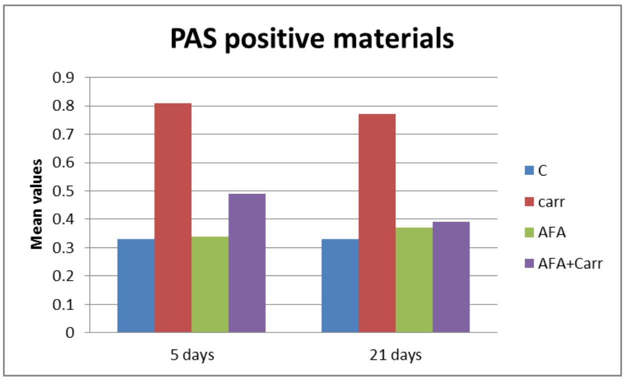


Figure 11- Showing the changes in PAS positive materials in sections of the liver tissue of the control and the treated groups after five and twenty one day post-treatment. Carrageenan group exhibited a significant increase in PAS positive materials all over the experimental periods.. While, rats administrated AFA alone and AFA post-carrageenan injection exhibited non-significant changes in PAS positive materials relative to the control group after five and twenty one days post-treatment**.**

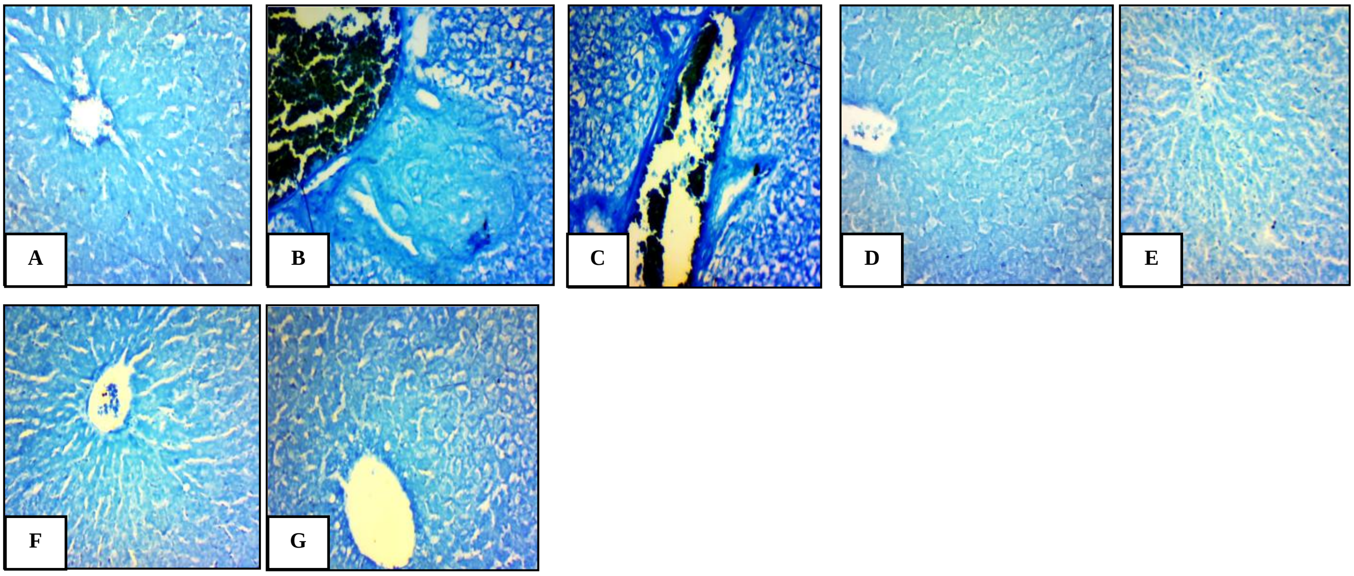


Figure 12- A. A photomicrograph of section of the liver tissue of the control group showing normal protein content in the portal area. **B,C.** Photomicrographs showing densely stained total protein in the liver tissue of carrageenan group (Carr) after five (B) and twenty one days(C). This increase was observed around highly dilated and congested hepatic portal veins, elongated and distorted walls of the bill ducts and around the vacuolated hepatocytes. **D,E.** Photomicrographs of sections of the liver tissue of AFA group showing normal protein content all over the liver tissue after five (D) and twenty one days (E). **F,G.** Photomicrographs showing somewhat normal distribution of total protein in the liver tissue of AFA+ Carr group as compared to the control group after five(F) and twenty one days (G),(X300).

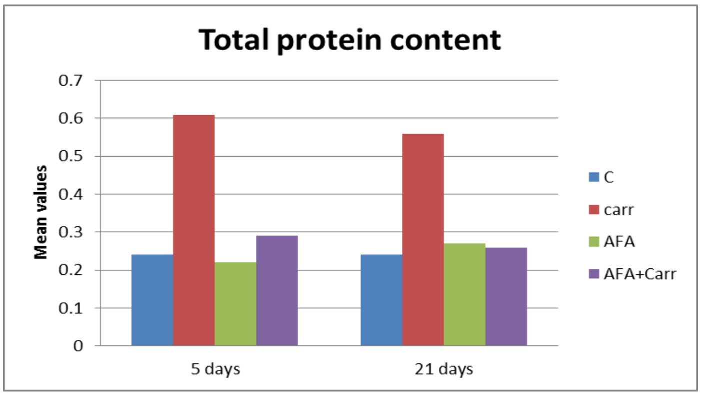


Figure 13- Showing the changes in total protein content in sections of the liver tissue of the control and all the treated groups after five and twenty one days post-treatment. Carrageenan group exhibited a significant increase in the total protein content in the liver tissue relative to the control group all over the experimental periods. Rats administrated AFA alone and AFA post-carrageenan injection exhibited non-significant changes in total protein content relative to the control group after five and twenty one days post-treatment.

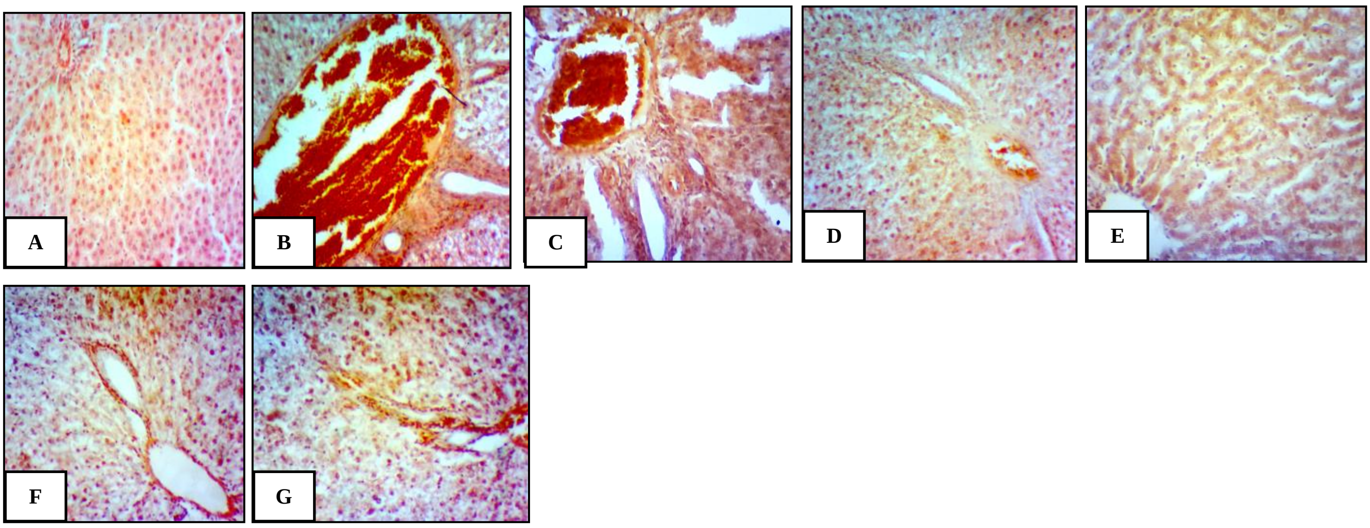
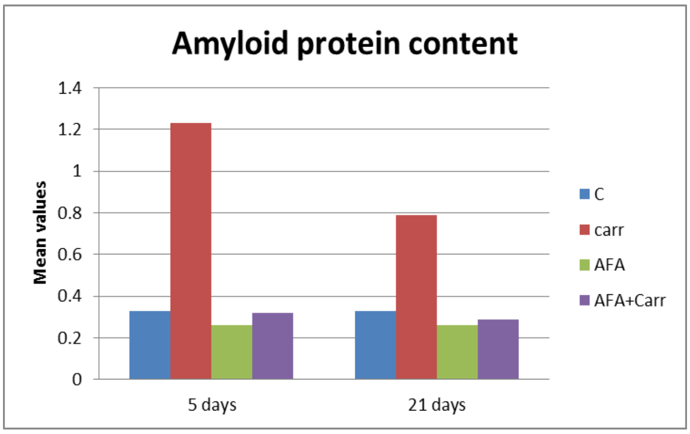


Figure 14- A. A photomicrograph of section of the liver tissue of the control group showing faintly stained amyloid protein. **B,C.** Photomicrographs showing deeply stained amyloid protein in the portal areas of the liver tissue of carrageenan group after five (B) and twenty one days (C). This increase was observed inside and around the highly dilated and congested hepatic portal veins, around elongated and distorted walls of the bill ducts. **D,E.** Photomicrographs of sections of the liver tissue of AFA group showing faintly stained amyloid protein all over the liver tissue after five (D) and twenty one days (E). **F,G.** Photomicrographs showing somewhat normal distribution of amyloid protein in the liver tissue of AFA+ Carr group as compared to the control group after five (F) and twenty one days (G),(X300).



Figures 15- Showing the changes in amyloid protein content in sections of the liver tissue of the control and all the treated groups after five and twenty one days post-treatment. Carrageenan group exhibited a significant increase in amyloid protein content in the liver tissue relative to the control group all over the experimental periods. While rats administrated AFA alone and AFA post-carrageenan injection exhibited non-significant changes in amyloid protein content relative to the control group after five and twenty one days post-treatment.

4 Discussion

In the present study, there were significant decreases in the mean values of RBCs, Hb and Hct of rats after five and twenty one days post-carrageenan injection as compared to the control group. These results come in agreement with those of Davidson *et al.* (1981) who reported that intraperitoneally injection of carrageenan (especially kappa and lambda) caused thrombocytopenia and red-cell damage within 2 days. This was followed by rebound thrombocytosis and persistent anemia (within 4 days) accompanied by a reticulocytosis. They also demonstrated that carrageenan exert several and varied effects on the haemopoietic system and its different cell lineages. Abdel-Raouf (2006) demonstrated that the decrease in hemoglobin and RBCs which is known as anemia, may be attributed to the effect of carrageenan on the haemopiotic system. In the present study supplementation of carrageenan injected group with AFA ameliorated the changes in the RBCs count, Hb and Hct. These findings are in agreement with those of Jenesn *et al.* (2001) ; Zhang *et al*. (2001) and Selmi *et al*. (2011) who reported that BGA prevent anemia and many other symptoms of nutritional deficiency because they give the body many nutrients which difficult to obtain them from other sources. Also, BGA have a stimulatory action on the metabolism of iron and hemoglobin in normal rats, thus reduce the severity of anemia and increase blood hemoglobin concentrations.

Results of the present study showed a significant elevation in white blood cells count of carrageenan injected group as compared to the control group. Similarly, Thomson and Fowler (1981) and Abdel-Raouf (2006) showed that carrageenan acts as a natural chemical substance capable of inducing production or proliferation of total leucocyte count in rats injected with carrageenan. Results of the present study illustrated that supplementation of rats with AFA alone and AFA post-carrageenan injection for twenty one days showed non-significant increase in the leucocytic count. Joshi *et al.* (2002) reported that BGA increased WBC count and this increase can be correlated with increased antibody production. Also, Sharma and Sharma (2005) reported that this stimulatory action of BGA might be attributed to its constituent phycocyanin which regulates the production of white blood cells even when bone marrow stem cells are damaged by toxic chemicals or radiation.

Results of the present study revealed that injection of rats with carrageenan significantly increased ALT, AST and ALP activities in serum when compared to the control group; this incidence indicated the liver damage by carrageenan. Significant elevation in liver enzymes after injection with carrageenan come in agreement with the results of Abdel-Raouf (2006) who showed that the liver enzymes (AST, ALT and ALP) were markedly elevated by the injection of carrageenan. The author also added that AST and ALT are intracellular enzymes involved in amino acid and carbohydrate metabolism. Elevation in concentrations of these enzymes in the blood indicated necrosis or disease in the tissue even before clinical symptoms of disease appear (Al-Menoufy, 2002).

The current study revealed that the liver functions have not been affected after oral administration of rats with 94.5 mg/kg of AFA daily for twenty one days as compared to those of the control group. These results go in parallelism with those of Mohamed *et al*. (2014) who showed that there is no significant change in the liver enzyme activities (ALT, AST and ALP) after oral administration of mice treated with 100 mg/kg of BGA (*Aphanizomenon flos-aquae*) for two weeks as compared to those of the normal control group. Treatment of rats with AFA post-carrageenan injection ameliorated the increases in ALT, AST and ALP activity. This comes in agreement with the work of Gini and Kurup (2010) who realized a significant increase in the activity of serum enzymes AST ,ALT and ALP in rats administrated paracetamol as compared to the control group. However, the activities of these enzymes were significantly lowered in rats treated with AFA (100 mg/ kg body weight) post-paracetamol administration in comparison with paracetamol administrated group. Furthermore, Sharoud (2015) reported that the presence of blue pigment phycocyanin in the blue green algae reduced the hepatotoxicity caused by paracetamol. Reduction in the levels of the liver enzymes induced by blue green algae attributed to the inhibition of reaction involved in the formation of reactive metabolites, cirrhosis and its radical scavenging activity.

In the present study examination of the liver tissue after five and twenty one days in the carrageenan injected group of male adult albino rats showed different histopathological alterations.These results are in agreement with those described previously by Kumar *et al.* (2014) who observed that in carrageenan injected group, the liver tissue showed dilation of the arteries and veins in the portal areas with thickening of the bile ducts epithelium second day post-injection. The heapatocytes were moderately swollen with prominent margins and nuclear karyorrhexis. On the fourth day after the injury, liver tissue showed micro-hemorrhagic spots. They also added that the histopathological study showed marked dilation of arteries and thickening of the bile ducts which revealed that carrageenan may act as a vasodilator. This effect of vasodilation could easily be observed under histopathological examination characterized by focal to diffuse hemorrhages in the capillaries of liver tissues at some places. The liver tissue study revealed edematous hepatocytes with marked vacuolation at places and also moderate degenerative changes.

Supplementation of rats with AFA post-carrageenan injection showed somewhat normal appearance of the central and portal areas of the liver tissue after five days, but some hepatocytes contained pyknotic nuclei with lymphocytic infiltration in and around the portal area and around the central vein. While, examination of sections of the liver tissue after twenty one days showed improvement of architecture of the liver tissue with well-developed central and portal areas. Karadeniz *et al*. (2008) and Ou *et al*. (2010) concluded that BGA protecting the liver from toxic injury of carbon tetra chloride (CCl_4) and preserving the hepatocyte ultrastructure and suggested that the probable hepatoprotective activity in rats might be due to the following effects: stabilizing the hepatocyte membrane by preventing lipid peroxidation, ameliorating the activities of the antioxidant enzymes, inhibition of the inflammation and the radical scavenging activity. These effects might be attributed to the increased amounts of phycocyanine and phenolic compounds and the antioxidant capacity as confirmed by three different antioxidant activity tests. They also added that phycocyanine could block inflammatory infiltration through its anti-inflammatory activities by inhibiting transforming growth factor-beta1 and hepatocyte growth factor expression. Also, Gini and Kurup (2010) reported that the congestion and inflammatory cell infiltration evoked by acetaminophen was considerably decreased by ethanol extract of AFA indicating its possible anti-hepatotoxic action. They also added that ethanol extract of AFA prevent hepatic injury by neutralizing oxidative stress. The hepatoprotective effect of AFA may be due to the presence of phycocyanin pigment present in it. Thus the possible mechanism of the hepatoprotective effect of ethanol extract of AFA is due to its antioxidant effect.

The present results are also in agreement with those of Alam *et al*. (2013) who observed that the presence of β-carotene, enzyme superoxide dismutase, vitamins or selenium in blue green algae produced an immune-stimulant activities and protective effects against paracetamol – induced liver damage. Also, Abdel-Daim *et al*. (2013) reported that the hepato-protective effect of BGA may be referred to its active components; β-carotene, blue pigment phycocyanin, linolenic acid, sulfated polysaccharide, vitamins (C and E) and selenium which provoke the activity of free radical scavenging enzyme system that render hepatic protection.

Results of the present study showed highly increased collagen fibres in and around the portal areas, inside the hepatic portal vein and also beside the central veins after five and twenty one days post- carrageenan injection.

Ramadori and Saile (2004) and Saile and Ramadori (2007) showed that hepatic stellate cells produce extracellular matrix and collagen when activated during inflammatory condition. In the current study treatment with AFA alone and AFA post-carrageenan injection showed somewhat normal distribution of collagen fibres around the hepatocytes, around the central veins, in the portal areas and in the blood sinusoids after five and twenty one days.

The obtained results showed that carrageenan group exhibited a significant increase in PAS positive materials all over the experimental periods. While, rats administrated AFA alone and AFA post-carrageenan injection exhibited non-significant increase in PAS positive materials relative to the control group after five and twenty one days post-treatment. Barsanti and Gualteri (2006) showed that AFA like other cyanobacter and plants uses photosynthesis to produce glycogen that is stored and utilized by the cell. While cell walls of plants are mainly cellulose, AFA's cell walls are composed of peptides and carbohydrates, the typical cell wall material of bacteria.

The present results revealed that carrageenan group exhibited a significant increase in the total protein content in the liver tissue relative to the control group all over the experimental periods. Borthakur *et al.* (2012) stated that carrageenan-induced increase in BCL10 protein expression (B-cell leukemia/lymphoma 10) ; total cellular BCL10 protein content remained significantly elevated compared to the control for 24 h after carrageenan withdrawal. One mechanism, proposed by the *in vitro* data, is that carrageenan binds to a membrane receptor known as Toll-Like-Receptor 4 (TLR4) and triggers a signaling cascade resulting in the expression of proinflammatory cytokines (Bhattacharyya *et al,* 2008a, b). Rats administrated AFA post-carrageenan injection exhibited non-significant changes in total protein content relative to the control group after five and twenty one days post-treatment. Stengel *et al*. (2011) demonstrated that cyanobacteria contain phycobiliproteins, a group of proteins involved in photosynthesis. Pigments such as phycobilin and phycoerythrin are associated with these proteins and these compounds have been found to have hepatoprotective, anti-inflammatory, immunomodulating, anticancer and antioxidant properties.

The present study showed increased amyloid protein content in the liver tissue of carrageenan group relative to the control group all over the experimental periods. While, treatment with AFA post-carrageenan injection showed somewhat normal deposition of amyloid protein content relative to the control group after five and twenty one days post-treatment. According to Hwang *et al.* (2011) treatment with 200 mg/kg of BGA *(Spirulina)* water extract decreased lipid peroxide and amyloid β-protein deposition in the brain of senescence-accelerated mice.

5 References

Abdel-Daim MM, Abuzead SMM, Halawa SM (2013). Protective role of *Spirulina platensis* against acute deltamethrin-induced toxicity in rats. PLoS ONE 8(9): e72991. doi:10.1371/journal.pone.0072991

Abdel-Raouf, O.M. (2006). Impact of an anti-inflammatory drug combined with heat stress on certain haematological, biochemical and histological parametars. Ph. D. Thesis, Zoology Department, Faculty of Science, Al-Azhar University.

Alam,M.A.; Haider,N.; Ahmed,S. ; Alam,M.T. ; Aziz,A. and Perveen,A. (2013).Tahlab (*Spirulina*) and few other medicinal plants having anti-oxidant and immunomodulatory properties described in unani medicine- a review. Int. J. Pharmaceut. Sci. Res., 4: 4158-4164.

Al-Menoufy,G.A. (2002). Effect of repeated administration of some anti-inflammatory drugs on the pathological activity of albino rats.Ph.D. Thesis, Zool. Dep., Faculty of Sc., Cairo University.

Bancroft,J.D. and Gamble,M. (2002). Theory and Practice of Histological Techniques. 5th ed., Churchill Living Stone. London, pp: 150-152.

Barsanti,L. and Gualteri,P. (2006). Algae Anatomy, Biochemistry and Biotechnology. C.R.C. Press. London, pp:1467-1471.

Benedetti,S. ; Benvenuti,F. ; Pagliarani,S. ; Francogli,S. ; Scoglio,S. and Canestrari,F. (2004). Antioxidant properties of a novel phycocyanin extract from the blue-green alga *Aphanizomenon flos-aqua*e. Life Sci., 75(19):2353-2362.

Benedetti,S.; Benvenuti,F.; Scoglio,S. and Canestrari,F. (2010). Oxygen radical absorbance capacity of phycocyanin and phycocyanobilin from the food supplement *Aphanizomenon flos- aqu*ae. J. Med. Food, 13(1):223-227.

Bhattacharyya,S.; Dudeja,P.K. and Tobacman,J.K. (2008a). Lipopolysaccharide activates NF-kB by TLR4-Bcl10-dependent and independent pathways in colonic epithelial cells. Amer. J. of Physiol., 295:784–790

Bhattacharyya,S. ; Ravinder,G. ; Chen,M.L.; Zhang,F. ; Linhardt,R.J. ; Dudeja,P.K. and Tobacman,J. K. (2008 b).Toll-like receptor 4 mediates induction of the Bcl10-NFκB-interleukin-8 inflammatory pathway by carrageenan in human intestinal epithelial cells. J. Biol. Chem., 238:10550–10558.

Borthakur,A.;Bhattacharyya,S.;Anbazhagan,A.N.; Kumar, A. ; Pradeep,K. ; Dudeja,P.K. ; Joanne,K. and Tobacman,J.K. (2012). Prolongation of carrageenan-induced inflammation in human colonic epithelial cells by activation of an NFκB-BCL10 loop. Biochem. Biophys. Acta. Molecul. Basis of 1822(8):1300-1307.

Chakdar,H. ; Jadhav,S.D. ; Dhal,D.W. and Pabbi,S. (2012). Potential applications of blue green algae. J. Sci. Indust. Res. , 71: 12-30.

Dacie,J.V. and Lewis,S.M. (1991). Practical Haematology. 7th ed., Churchill Livingstone. Edinburgh, pp: 48-52.

Davidson,R.J.L. ; Avidson,J.G. ; Simpson,P.H. ; Whiting,J.I. ; Milton,J.I. and Thomson,A.W. (1981). Haematological changes following systemic injection of purified carrageenans (Kappa, Lambda and Iota). Br. J. Exp. Path., 62: 529-536.

Drabkin,D.L. and Austin,J.H. (1932). Spectrophotomeric studies: Spectrophotometric constant for common hemoglobin derivatives in human, dogs and rabbit blood. J. of Biolog. Chem., 98: 719-725.

Drury,R. and Wallington,E. (1980). Carleton's Histological Technique. 4th ed., Oxford. Univ. Press, New York.

El- Depsi,S.M. (2016). Evolution of the role of glibenclamid and *Aphanizomenon flos-aquae* extract on some organs of the induced diabetes rats. M.Sc. Thesis, Zoolgy Department, Faculty of Science, Al-Azhar University.

Ghosh,A.K.;Hiraswa,N.; Niki,H. and ohuchi,K. (2000). Cyclo-oxygenase to mediated angiogenesis in carrageenan induced granulation tissue in rats. J. Pharmacol. Exp.Therap., 295(2): 802 – 809 .

Gini,C.K. and Kurup,M.G. (2010). Antioxidant and hepatoprotictive activity of *Aphanizomenon flos-aquae* line against paracetamol intoxication in rats. Indian J. Exper. Bio., 48:1123-1130.

Hwang,J.H. ; Lee,I.T. ; Jeng,K.C.; Wang,M.F. ; Hou,R.C. ; Wu,S.M. and Chan,Y.C. (2011). *Spirulina* prevents memory dysfunction, reduces oxidative stress damage and augments antioxidant activity in senescence-accelerated mice. J. Nutr. Sci. Vitaminol., 57:186–191.

Jensen, G.S. ; Ginsberg, D.I. and Drapeau, C. (2001).Blue green algae as an immuno- enhancer and biomodulator. J. Amer. Nutraceut. Assoc., 3: 24-30.

Joshi, P. ; Harish, D. and Bose, M. (2002). Effect of lindane and malathion exposure to certain blood parameters in a fresh water teleost fish *Clarias batrachus*. Poll. Res., 21:55-57.

Karadeniz, A. ; Yildirim, A. ; Simsek, N. ; Kalkan, Y. and Celebi, F. (2008). *Spirulina platensis* protects against gentamicin-induced nephrotoxicity in rats. Phytother. Res., 22: 1506–1510.

Kumar, V. ; Kumar, K. ; Raman, R. P. ; Prasad, K.P. ; Roy, S. ; Kumar, S. and Kumar, N. (2014). Haematological and histopathological changes during carrageenan induced acute inflammatory response in labeorohita (Hamilton, 1822) fingerlings. Int. J. Curr. Microbiol. App. Sci., 3(7): 794-802.

Mazia,D. ; Brewer,P.A. and Alfert,M. (1953). The cytochemical staining and measurement of protein with mercuric bromophenol blue. Biol. Bull., 104:57-67.

Mc-Kim, J.M. (2014). Food additive carrageenan part I: a critical review of carrageenan *in vitro* studies, potential pitfalls and implications for human health and safety. Crit. Rev. Toxicol., 44(3): 211–243.

Mitruka,B.M. and Rawnsley,H.M. (1977). Clinical Biochemical and Haematology Reference Values in Normal and Experimental Animals. New York, Masson Publishing, USA.

Mohamed, A.H. ; Osman, G.Y. ; Salem, T.A. and Elmalawany, A.M. (2014). The hepatoprotective activity of blue green algae in *Schistosoma mansoni* infected mice. Exp. Parasitol., 145:7-13.

Nahin, R.L. ; Barnes, P.M. ; Stussman, B.J. and Bloom, B. (2009).Costs of complementary and alternative medicine (CAM) and frequency of visits to CAM practitioners. Natl. Health Stat. Rep., 30(8):1–14.

Ou, Y. ; Zheng, S. ; Lin, L. ; Jiang, Q. and Yang, X. (2010). Protective effect of C-phycocyanin against carbon tetrachloride-induced hepatocyte damage *in vitro* and *in vivo*. Chem. Biolog. Interact., 185(2):94–100.

Paget, E. and Barnes, M. (1964). Interspecies dosage conversion scheme in evaluation of results and quantitative application in different species. Evaluat. Drug Activities Pharmacometric, 1: 160-162.

Pears, A. (1977): Histochemistry Theoretical, and Applied. 3rd ed., Vol. 1. Churchill Livingstone, London.

Ramadori, G. and Saile, B. (2004). Portal tract fibrogensis in the liver. Lab. Invest., 84: 153-159.

Reddy, C.M. ; Bhat, V.B. ; Kiranmai, G. ; Reddy, M.N. ; Reddanna, P. and Madyastha, K.M. (2000). Selective inhibition of cyclooxygenase-2 by C-phycocyanin a biliprotein from *Spirulina platensis*. Biochem. Biophys. Res. Commun., 277 (3): 599–603.

Regunathan,C. and Wesley,S.G. (2006). Pigment deficiency correction in shrimp broodstock using *Spirulina* as a carotenoid source. Aquacult. Nutr., 12:425–432.

Rodak,L.C. (1995). Routing testing in haematology. In: Dignostic Haematology. W.B. Saunders Comp. Philadelphia London, Toronto, pp: 128-144.

Saile,B. and Ramadori,G. (2007). Inflammation damage repair and liver fibrosis role of cytokines and different cell types. Gastroenterol., 45:77-86.

Schaap,A. ; Rohrlack,T. and Bellouard,Y. (2012). Optical classification of algae species with a glass lab on a chip. Lab. Chip. J., 12:1527-1532.

Schumann,G. ; Klauke,R. ; Canalias,F. ; Bossert-Reuther,S. ; Franck,P.F. and Gella,F.J. (2002). IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 C. Clin. Chem. Lab. Med., 40(7):718-724.

Scoglio,S. ; Benedetti,Y. ; Benvenuti,F. ; Benedetti,S. and Benedetti,S. (2014). Selective monoamine oxidase B inhibition by an *Aphanizomenon flos-aquae* extract and by its constitutive active principles phycocyanin and mycosporine-like amino acids. Phytomedicine, 21(7):992-997.

Scoglio,S.; Benedetti,S.; Canino,C.; Santagni,S.; Rattighieri, E.; Chierchia,E.; Canestrari,F. and Genazzani, A.D. (2009). Effects of a 2-month treatment with Klamin, a Klamath algae extract, on the general well-being, antioxidant profile and oxidative status of postmenopausal women. Gynecolog. Endocrinol., 25: 235–240.

Selmi,C. ; Leung,P.S. ; Fischer,L. ; German,B. ; Yang,C.Y. ; Kenny,T.P.; Cysewski,G.R. and Gershwin,M.E. (2011). The effects of *Spirulina* on anemia and immune function in senior citizens. Cell Mol. Immunol., 8:248–254.

Sharma, S. and Sharma, S. (2005). Protective role of *Spirulina* feed in a fresh water fish (*Poecilia reticulata*) exposed to an azo dye-methyl red. Indian J. Exp. Biol., 43: 1165-1172.

Sharud, M.N.M. (2015). Protective effect of *Spirulina* against paracetamol induced hepatic injury in rats. J. Exp. Biol. Agricul. Sci., 3(1):34-44.

Singh,S.; Kate,B.N. and Banerjee,U.C. (2005).Bioactive compounds from cyanobacteria and microalgae: an overview. Crit. Rev. Biotechnol., 25:73–95.

Sini,J.M.; Yaro,A.H.; Ayanwuyi,L.O.; Aiyelero,O. M.; Mallum,S.M.and Gamaniel,K.S. (2010): Anti-nociceptive and anti-inflammatory activities of the aqueous extract of the root bark of Combretum sericeum in rodents. African J.Biotechnol., 9: 8872–8876.

Stengel,D.B. ; Connan,S. and Popper,Z.A. (2011).Algal chemodiversity and bioactivity: sources of natural variability and implications for commercial application. Biotechnol. Advanc., 29(5):483–501.

Thomson, A.W. (1981). Haematological changes following systemic injection of purified carrageenans (Kappa, Lambda and Iota). Br. J. Exp. Path., 62: 529-536.

Thomson,A.W. and Fowler, E.F. (1981). Carrageenan: a review of its effect on the immune system. Agents Action,1: 265-273.

Tiniakos,D.G. ; Vos,M.B. and Brunt,E.M. (2010). Nonalcoholic fatty liver disease: pathology and pathogenesis. Ann. Rev. Pathol., 5:145-171.

Valle,S. (1986). Special stains in microwave oven. J. Histotechnol., 9:237-248.

Venkatesan,S.;Pugazhendy,K.;Meenambal,M.; Sangeetha,D.; asantharaja,C.V. ; Jayachandren,K. and Prabakaran,S. (2012). Protective role of *Spirulina* on the variation of haematological parameter induced by herbicide atrazine in the fresh water fish *Cyprinus carpio* (Linn). Int. J. Pharm. Biol. Arch., 3:249-254.

Weiner,M.L. (2014). Food additive carrageenan, Part II: a critical review of carrageenan *in vivo* safety studies. Crit. Rev. Toxicol., 44(3):244-269.

Yang,Y.;Park,Y.;Cassada,D.A.;Snow,D.D.; Rogers, D.G. and Lee,J. (2011). *In vitro* and *in vivo* safety assessment of edible blue-green algae, *Spirulina plantensis*. Food Chem. Toxicol., 49(7):1560-1564.

Yeh,G.Y. ; Davis,R.B. and Phillips,R.S. (2006).Use of complementary therapies in patients with cardiovascular disease. Am. J. Cardiol., 98:673–680.

Young, D. and Friedman, R. (2001). Effects of Disease on Clinical Laboratory Tests. 4th Edition, A.A.C.C. Press, Washington, USA.

Zhang,H.Q.;Lin,A.P.; Sun,Y. and Deng,Y.M. (2001). Chemo- and radioprotective effects of polysaccharide of *Spirulina platensis* on hemopoietic system of mice and dogs. Acta. Pharmocol. Sin., 22: 1121-1124.