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

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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. It has been traditionally used for over 25 years for its health-enhancing properties**.** *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**. Aim of the study:** this study aimed to examine the possible anti-inflammatory effect of AFA against the inflammation induced by carrageenan injection on skin of adult male rats using histpathological and histochemical studies.**Matrerial and methods**: the current experiment was carried out on 48 adult male albino rats (*Rattus rattus*). Rats were randomly and equally categorized into four groups: **1) Control group (C)**: rats were left without treatment; **2) Carr group**: rats were injected with 0.1ml of carrageenan and left for 21 days ; **3) AFA group:** rats were orally administrated *Aphanizomenon flos- aquae* (AFA) extract (94.5 mg/kg body weight /day) for 21 days and 4**) AFA+ Carr Group**: rats were injected with carrageenan and treated with 94.5 mg/kg body weight AFA extract daily after six hours of carrageenan injection for 21 days. The experimental rats were sacrificed after 5 and 21 days post– treatment. **Results:** Examination of skin tissue of rats five and twenty one day's post-carrageenan injection revealed many histopathological and histochemical changes such as marked destructed epidermal and dermal layers. The epidermal layer showed undetectable cellular structure, thickened keratin layer. Signs of fibrosis and absence of hair follicles were detected in some areas, in addition to the presence of debris of degenerated cells in the dermal layer. Hair follicles were distorted with numerous fibroblasts in the dermal layer, some of them were hypertrophied, in addition to the presence of large granulomatous area in the dermal layer, discontinuous and faintly stained skeletal muscle fibres were noticed. Most of them showed decreased staining affinity of nuclei of mycocytes (karyolysis) with signs of fatty degeneration. Highly increased collagen fibres and fibrotic areas were detected in the epidermal and dermal layers.

Skin tissues examined five and twenty one days following AFA administration showed normal appearance of the epidermal and dermal layers, highly increased and well developed hair follicles with their sebaceous glands were detected with normal distribution to some extent, of collagen fibres.

Skin tissues of rats administrated with AFA for twenty one days post-carrageenan injection and examined after five and twenty one days showed striking recovery as compared to the skin of carrageenan group only, but increased collagen fibres in the dermal layer were detected after five days while normal distribution of collagen fibres were demonstrated after twenty one days.

The quantitative histochemical measurements recorded a significant increase in PAS+ve materials , total protein and amyloid β -protein in the carrageenan injected group while supplementation with AFA alone or AFA post carrageenan injection showed a trend toward lowering incidence of skin histochemical changes induced by carrageenan injection. Skin tissues of carrageenan group showed a significant increase in mast cells count in the dermal layer after five and twenty one days post-treatment**.** AFA treated group exhibited non-significant increase of mast cells in the dermal layer all over the experimental periods, while rats administrated AFA post-carrageenan injection exhibited a significant increase in count of mast cells after five days and non-significant increase after twenty one days **Conclusion:** using *Aphanizomenon flos-aquae* as a natural agent exerted a marked anti-inflammatory role against the histopathological and histochemical lesions induced by carrageenan injection**.**

Keywords: carrageenan, *Aphanizomenon flos- aquae*, inflammation and rats.

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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)**. The edema induced by carrageenan is due, in part, to the liberation of bioamines. During the first hour, the principal agents implicated in the edema formation are the bioamines histamine , serotonin and the peptide bradykinin. These are followed in time by prostaglandins, which are the principal mediators involved in the increased vascular permeability together with nitric-oxid, which is formed by leukocytes, endothelial cells and sensory nerve cells at the site of inflammation. In the acute phase, the increase in vascular permeability produces cell infiltration, mainly neutrophils, increasing the inflammatory response after the production of oxygen-derived species **(Dash and Kanungo, 2013).**

Inflammation is a complex cellular and biochemical response to injurious stimuli that involve many cytokines and inflammatory mediators. It is a normal essential protective response to any noxious stimulus that may threaten the body and may differ from a localized reaction to a complex response involving the whole organism**(Cotran *et al*., 2004)**. Agents which modify inflammatory responses are called anti-inflammatory drugs. Non-steroidal anti-inflammatory drugs (NSAID) are generally used to treat acute and chronic inflammatory conditions. However, due to their adverse side-effects and increased cardiovascular disease (CVD) risk is associated with chronic use of several NSAID**(Jugdutt, 2007);** there is a critical need to identify natural products with anti-inflammatory properties.

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 **(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)**. **Selmi *et al*. (2011)** demonstrated that BGA consumption protects against inflammatory diseases and promotes immunity**.**They are nutritious natural products rich in essential amino acids, γ-linolenic acid, B vitamins, fibres, calcium, phosphorous, iron, 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 **(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 **(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 **(Benedetti *et al*., 2010).** Moreover, carotenoids can serve as marker for the whole antioxidant status of the human epidermis **(Haag *et al*., 2013).**

2 Material 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 design

The experimental animals were divided into 4 groups:

Group 1- Untreated normal control rats **(C).**

Group 2- Rats were injected with carrageenan 0.1 ml and left for 21 days **(Carr).**

Group3-Rats were orally administrated *Aphanizomenon flos- aquae* (AFA) extract (94.5 mg/kg body weight /day) for 21 days.

Group 4- Rats were injected with carrageenan (0.1ml) and treated with 94.5 mg/kg body weight AFA extract daily after 6 hours of carrageenan injection for 21 days (AFA+ Carr).

The experimental rats were sacrificed after 5 and 21 days post– treatment.

Inflammatory model (rat paw edema)

Carrageenan (Carr) was obtained from Sigma Company. Type IVEC No. 232-953-5. Carrageenan 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.

Preparation of *Aphanizomenon flos-aquae* (AFA) extract

AFA-Klamath capsules (350 mg) purchased from German Egyptian Pharmaceutical Company. AFA capsule were opened and dissolved in distilled water. The drug was

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administrated orally by gastric tube at a dose of 94.5 mg/kg body weight/day for 21 days. The dose for the rat was calculated according to the Paget's formula on the basis of the human dose **(Paget and Barns, 1964).**

-Histopathological and histochemical techniques

Skin of leg paw of all groups were washed in saline and fixed in 10% neutral formalin, followed by dehydration in ascending grades of alcohol, cleared in xylene and embedding in paraffin wax. Sections were then cut at 5µ thickness and stained by haematoxylin and eosin for histopathological study according to the method of **Bancroft and Gamble (2002).** Collagen fibres were stained by Mallory's trichrome **(Pears, 1977).** Polysaccharides were detected by using periodic acid Schiff's (PAS) reagent **(Drury and Wallington, 1980)**. Total proteins could be visualized by mercuric bromophenol blue method **(Mazia *et al.,* 1953)**. Mast cells were stained by toluidine blue (**Sheehan and Hrapchak, 1980).** Amyloid-β protein was detected by Congo red technique **(Valle, 1986).**

Quantitative histochemical analysis

The optical density of histochemical stained sections in skin tissue for carbohydrates, total protein and Amyloid-β protein of the control and treated groups as well as count of mast cells were recorded using IPWIN 32 image analysis software.

Statistical analysis

Statistical analyses were performed using analyses of variance (ANOVA) according to **Snedecor and Cochran (1980)**. The data were processed and analyzed using the SPSS software (Statistical Analysis for Social Science, Version 8). Significant differences between treatment means were determined by student t-test. Data were presented as mean ± SE and $P < 0.05$ was considered statistically significant.

3 Results

Histopathological and histochemical observation

The control group

The normal structure of leg skin of a control rat is demonstrated in **figs. 1 and 2.** The skin is mainly formed of the following layers

1- Epidermis, an epithelial layer formed of stratified keratinized squamous epithelium of ectodermal origin.

2- Dermis , a connective tissue layer of mesodermal origin. The dermis containing hair follicles, glands and receptors. A subcutaneous connective tissue layer (hypodermis) is found**.**

Normal distribution of collagen fibres in the epidermal and dermal layers is shown in **figs. 3 and 4**. Thin collagen fibres are supporting the epidermal and dermal layers.

Carrageenan injected group

Examination of skin tissue of rats five days post-carrageenan injection showed completely degenerated epidermal and dermal layers.

The epidermal layer showed undetectable cellular structure, thickened keratin layer with highly affected and distorted epidermal layer. Signs of fibrosis and absence of hair follicles were detected in some areas, in addition to the presence of debris of degenerated cells in the dermal layer. Hair follicles were distorted with numerous fibroblasts in the dermal layer, some of them were hypertrophied, in addition to the presence of large granulomatous area **(Figs. 5-8)**.

By the end of twenty one days following carrageenan injection , marked destructed epidermal and dermal layers were detected, in addition to absence of cellular structure of the corrugated epidermal layer. Highly distorted and reduced hair follicles with ruptured, discontinuous and faintly stained skeletal muscle fibres were noticed. Most of them showed decreased staining affinity of nuclei of mycocytes (karyolysis) with signs of fatty degeneration **(Figs. 11-15).**

Concerning distribution of collagen fibres, highly increased collagen fibres and fibrotic areas were detected in the epidermal and dermal layers after 5 **(Figs.9, 10**) and 21 days **(Figs. 16, 17)**.

AFA-treated group

Skin tissues examined five and twenty one days following AFA administration showed normal appearance of the epidermal and dermal layers, highly increased and well developed hair follicles with their sebaceous glands were detected**(Figs. 18,19)** with normal distribution of collagen fibres**(Figs. 20,21).**

Group treated with AFA post-carrageenan injection

Skin tissues of rats administrated with AFA for twenty one days post-carrageenan injection and examined after five and twenty one days showed striking recovery as compared to the skin of carrageenan group only, but increased collagen fibres in the dermal layer were detected after five days **(Figs. 22, 23, 25).**

As shown in **figs. 24 and 26** epidermal and dermal layers were resuming their normal structure after twenty one days with highly increased and well developed hair follicles in the expanded dermal layer and somewhat normal distribution of collagen fibres could be demonstrated.

Quantitative histochemical measurements

The present study revealed that the group of rats injected with carrageenan exhibited a significant increase in the mean value of PAS positive materials all over the experimental periods. While, rats administrated AFA alone or AFA post-carrageenan injection exhibited a non-significant increase in the mean value of PAS positive materials relative to the control group after five and twenty one days post-treatment. Carrageenan group exhibited a significant increase in the mean value of total protein while the group treated with AFA alone exhibited non-significant increase in the mean value of total protein all over the experimental periods. Rats administrated AFA post-carrageenan injection induced a significant increase in the mean value of total protein after five days while non significant increase after twenty one days could be recorded. Carrageenan group showed a significant increase in the mean value of amyloid β -protein content in skin tissue relative to the control group all over the experimental periods while, rats administrated AFA alone or AFA post-carrageenan injection induced a non-significant change in

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the mean value of amyloid β -protein during the two experimental periods.

Mast cells count

Figures 52- 58 illustrated the changes in count of mast cells in sections of the skin of the control(**Fig. 52)** and treated groups after five and twenty one days post-treatment.Skin sections of carrageenan group showed a significant increase in count of mast cells in the dermal layer after five and twenty one days post-treatment **(Figs. 53,54).** AFA treated group exhibited non-significant increase of mast cells in the dermal layer all over the experimental periods**(Figs. 55,56)**, while rats administrated AFA post-carrageenan injection exhibited a significant increase in count of mast cells after five days and non-significant increase after twenty one days **(Figs. 57-58)**.

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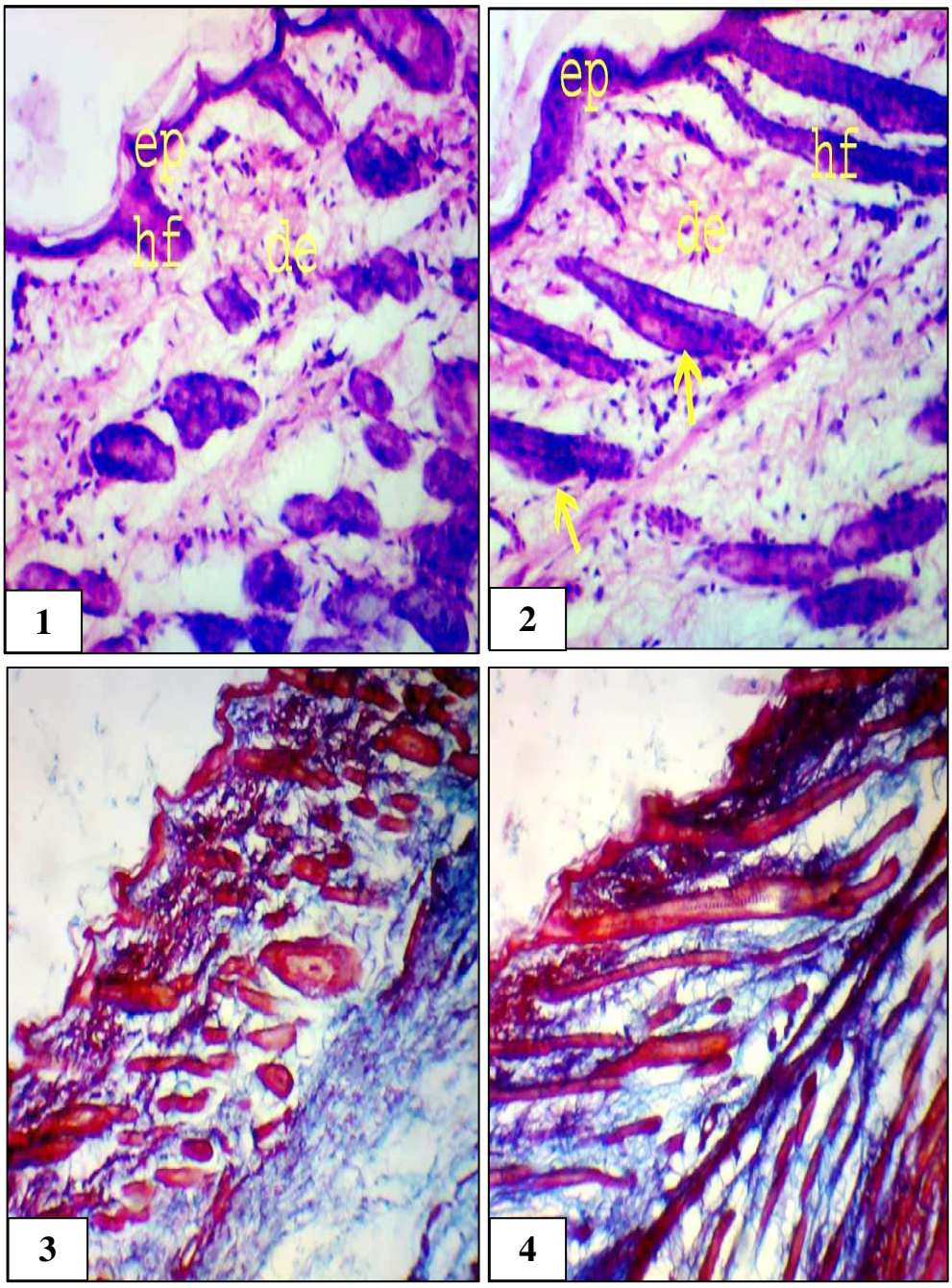

Figs. 1-26: photomicrographs of sections in skin tissue of the control and treated groups.
Figs. 1-4 : showing the skin tissue of the control group.
Figs. 1, 2:showing well developed epidermal **(ep)** and dermal layers **(de)** with hair follicles **(hf)** and sebaceous glands (→).**(H& E X100)**
Figs. 3, 4 : control rats showing numerous collagen bundles scattered in the epidermal and dermal layers.
(Mallory'strichrome stain x 100)

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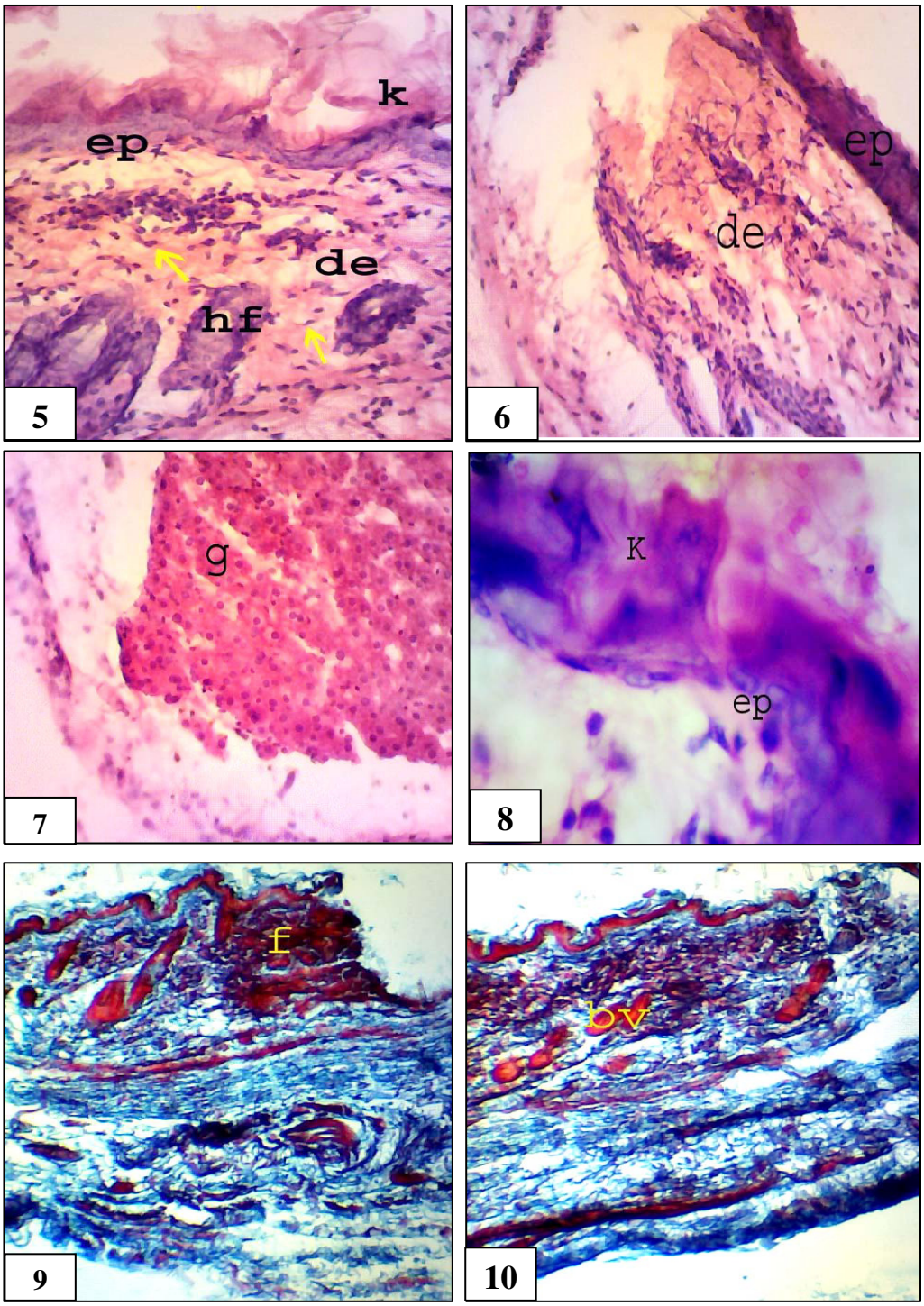

Figs. 5-10:photomicrographs of the skin tissue of rats five days post-carrageenan injection**.**

Figs. 5-8: showing distorted hair follicles **(hf)** with numerous fibroblasts (→), undetectable cellular structure of the epidermal layer **(ep)** with highly distorted and thickened keratin layer **(k)**, fibrosis and absence of hair follicles in the dermal layer **(de)**, presence of debris of degenerated cells and large granulomatous area **(g)** in the dermal layer**.**

(5- 7 H& E X100 ; 8 X400)

Figs. 9,10:showing highly increased collagen fibres in the epidermal and dermal layers.Notice: red brightly stained congested blood vessels **(bv)** and fibrotic areas **(f). (Mallory'strichrome stain X100)**

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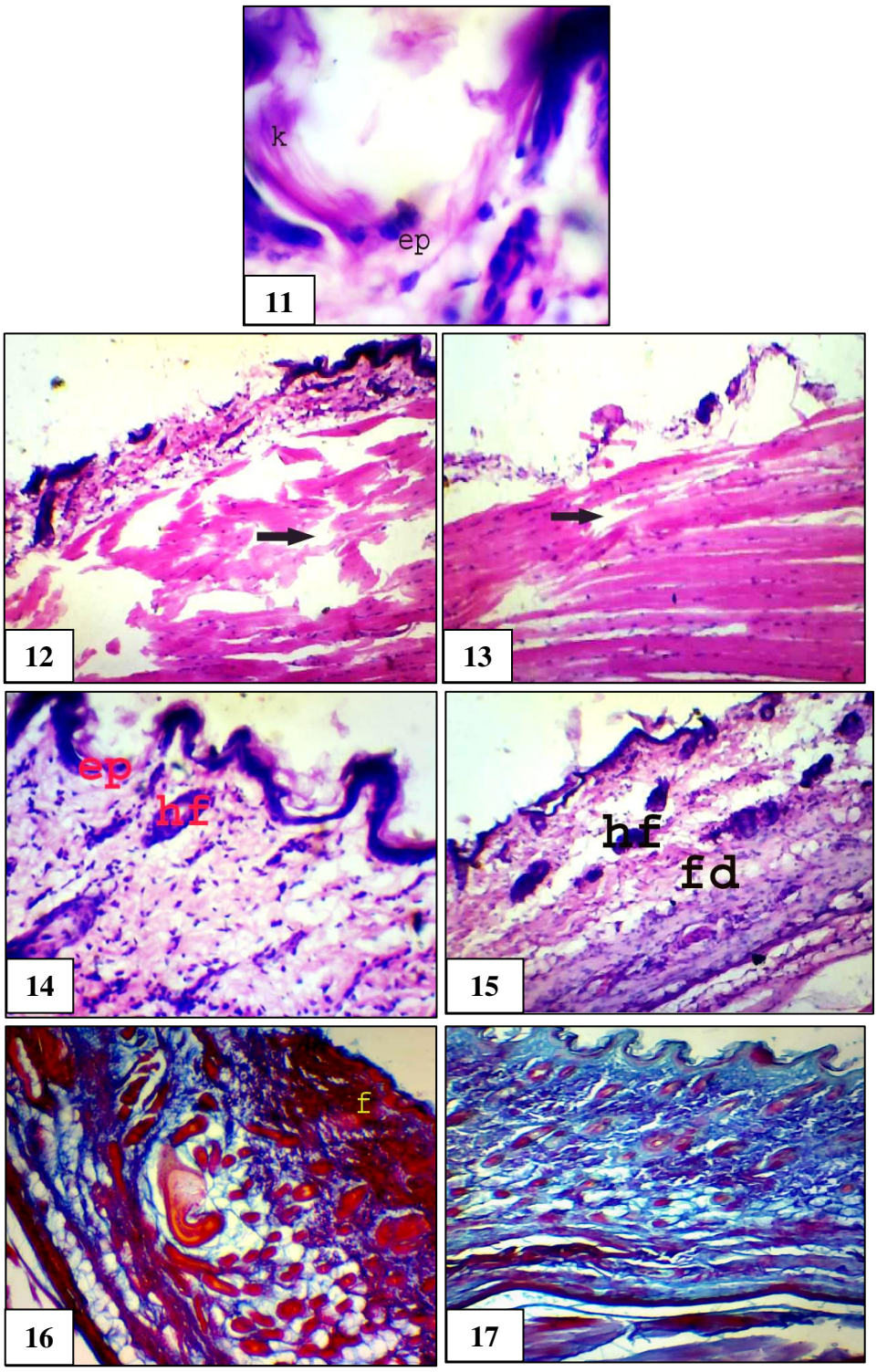

Figs. 11-17:photomicrographs of the skin tissue of rats twenty one days post- carrageenan injection.

Fig. 11:showing thickened keratin layer **(k)**with highly affected and distorted epidermal layer **(ep)**.**(H& E X400)**
Figs. 12, 13 :showing ruptured, discontinuous **(→)** and faintly stained skeletal muscle fibres.**(H& E X100)**
Figs. 14, 15 :showing absence of cellular structure of the corrugated epidermal layer, highly distorted and reduced hair follicles **(hf)** with signs of fatty degeneration **(fd)**.**(H& E X100)**
Figs. 16, 17:showing highly increased collagen fibres and fibrotic areas **(f)** in the epidermal and dermal layers.
(Mallory's trichrome stain X100)

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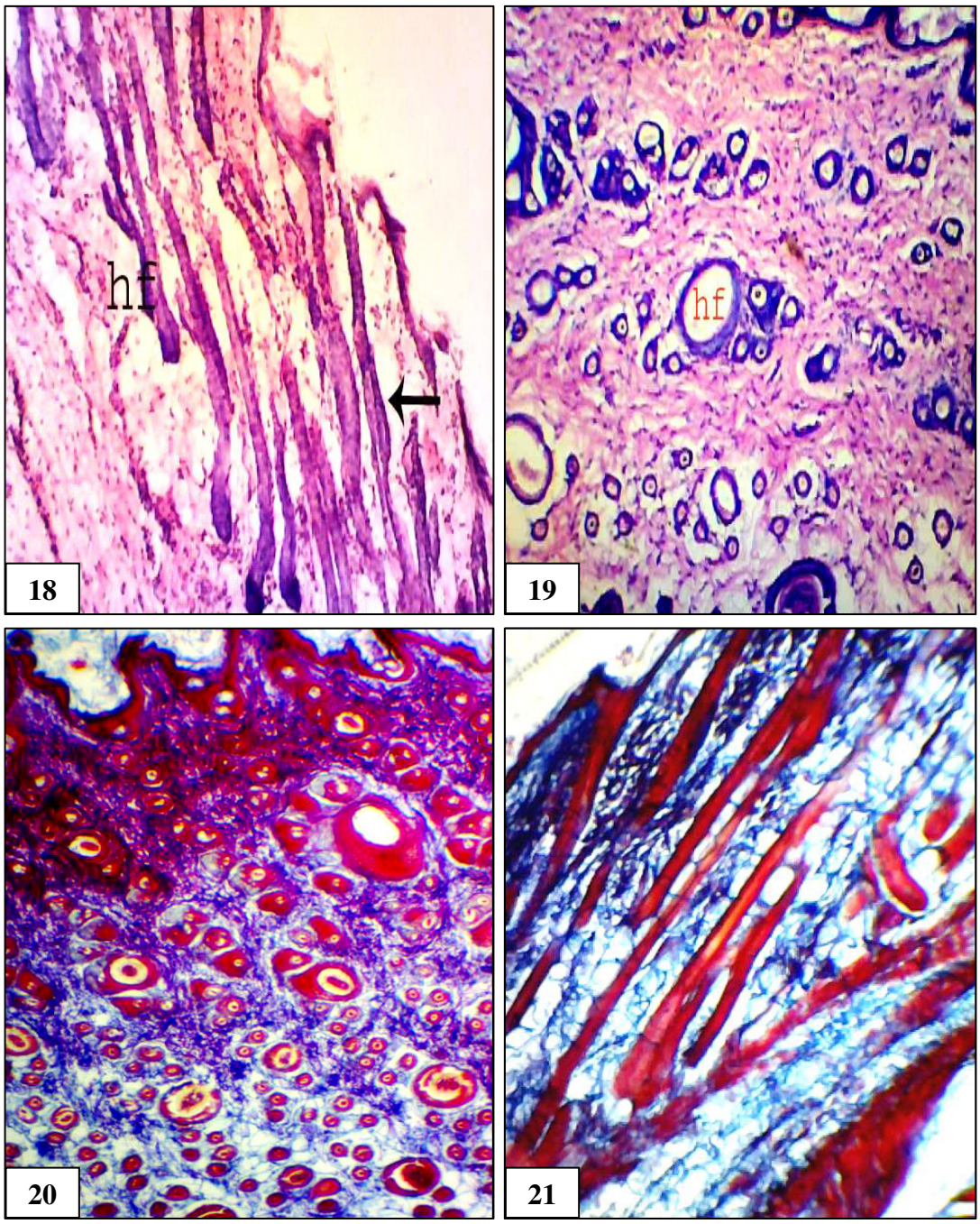

Figs. 18-21:photomicrographs of the skin sections of rats administrated AFA for five and twenty one days.
Figs. 18,19:showing normal appearance of the epidermal and dermal layers with highly increased and well developed hair follicles **(hf)** in the expanded dermal layer with their sebaceous glands **(→).(H & E X100)**
Figs. 20, 21 :showing normal distribution of collagen fibres in the epidermal and dermal layers after five **(20)** and twenty one days **(21)** . **(Mallory's trichrome stain X100)**

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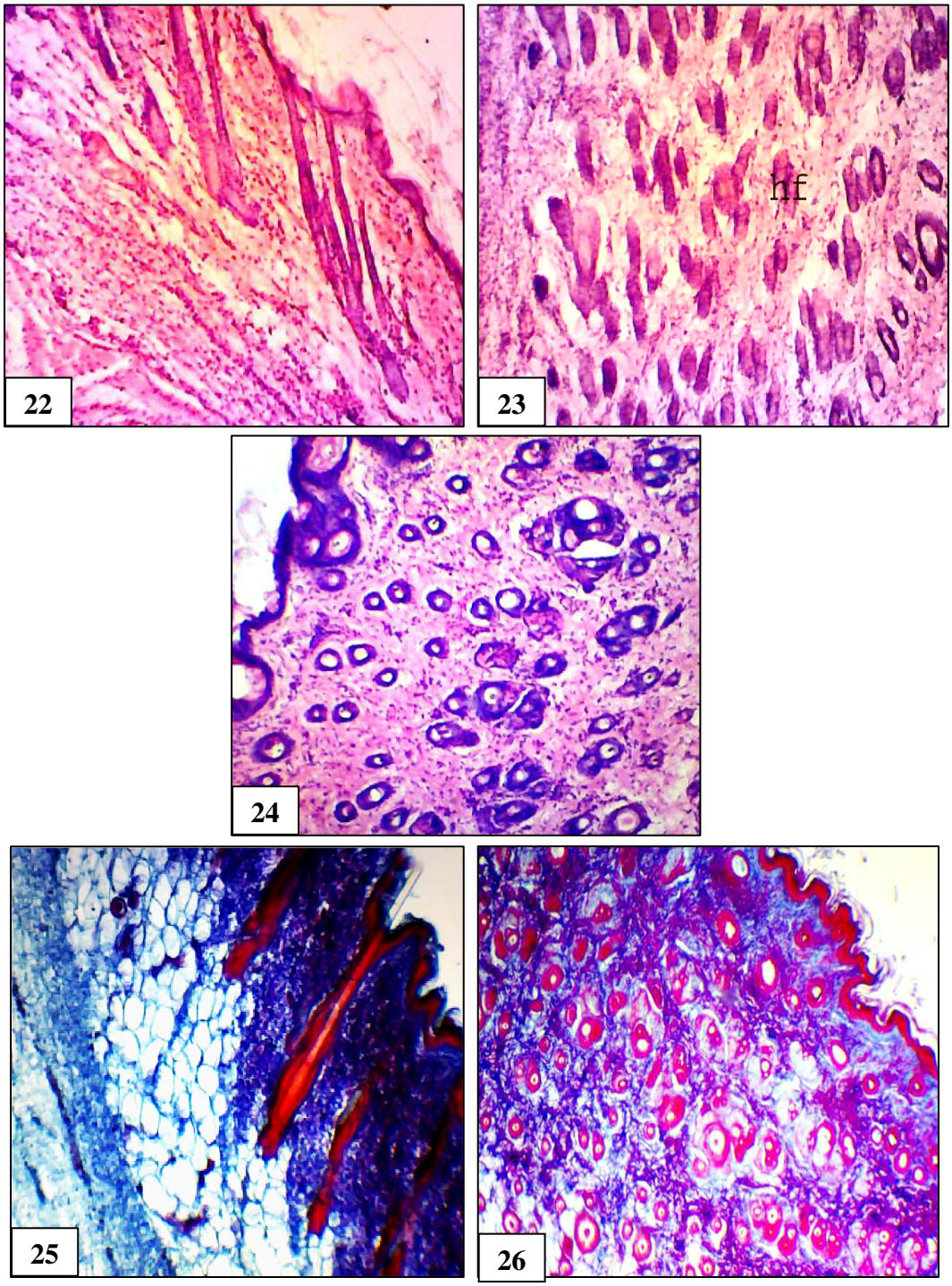

Figs. 22- 26 : photomicrographs of the skin sections of rats administrated AFA post-carrageenan injection for five and twenty one days.

Figs. 22, 23, 25 : showing somewhat normal appearance of the epidermal and dermal layers with slightly increased collagen fibres in the dermal layer after five days. **(29& 30 H & E X100 ;32 Mallory's trichrome stain X100)**

Fig. 24: showing normal structure of the epidermal and dermal layers with highly increased and well developed hair follicles in the expanded dermal layer after twenty one days. **(H& E X100)**

Fig. 26 : showing somewhat normal distribution of collagen fibres in the epidermal and dermal layers after twenty one days. **(Mallory's trichrome stain X100)**

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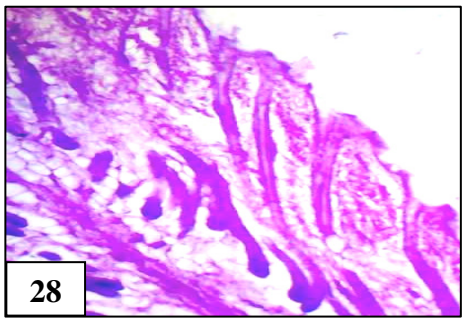
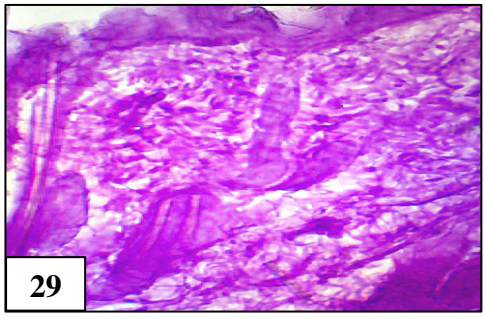
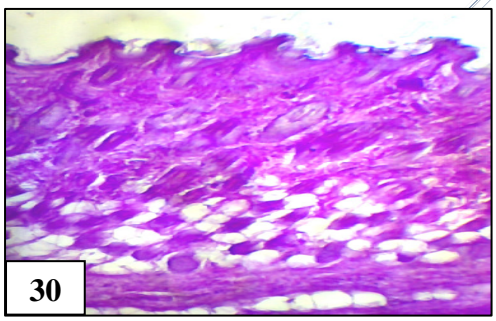
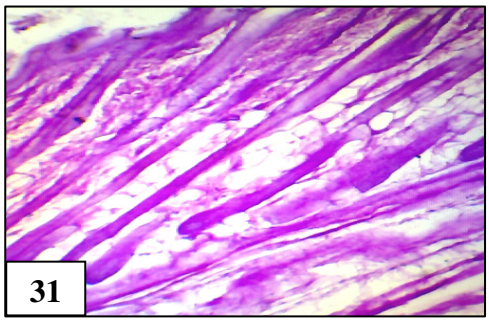
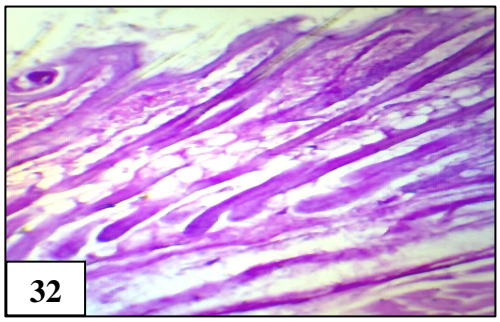
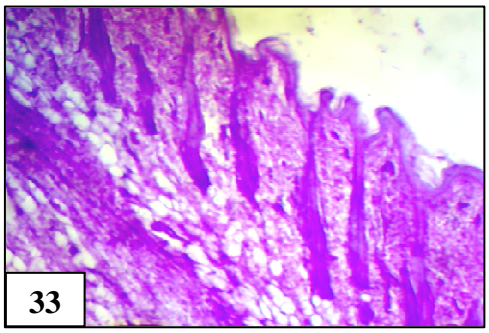


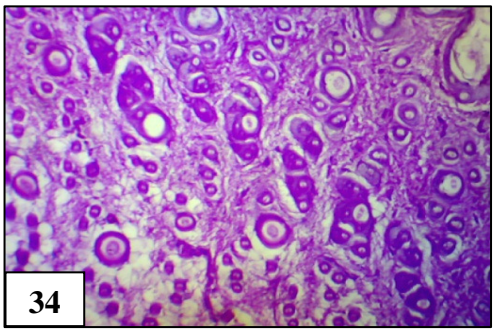












Figs. 28-34: photomicrographs of sections in skin tissue of the control and treated groups. (PAS)

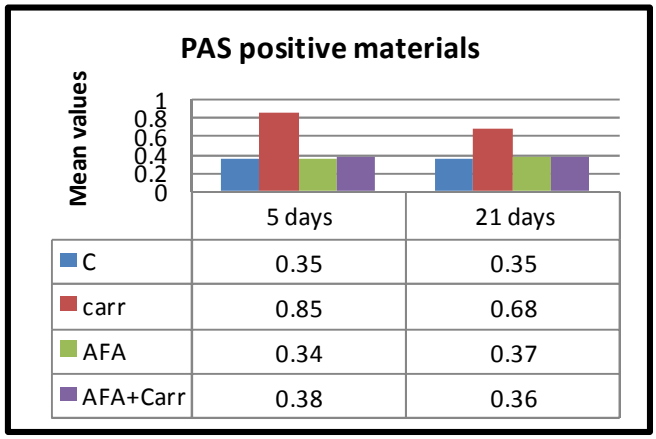
Fig. 28 A: photomicrograph showing normal distribution of polysaccharides in the epidermal and dermal layers of the skin of the control group.

Notice: moderately to deeply stained staining affinity in hair follicles and sub-dermal muscle fibres with less stained connective tissue. **(X 100)**

Figs. 29, 30 :photomicrographs showing increased content of polysaccharides in the corrugated epidermal and destructed dermal layers of the skin sections of carrageenan group after five **(Fig. 29)** and twenty one days **(Fig. 30)**. **(29 X 200 ; 30 X 100)**

Figs. 31,32:photomicrographs showing normal distribution of polysaccharides in the skin sections of AFA group after five **(Fig. 31)** and twenty one days **(Fig. 32)**. **(X 100)**

Figs. 33, 34:photomicrographs of sections of the skin tissue of AFA +Carr group showing slightly increased polysaccharides content after five **(Fig. 33)** and twenty one days **(Fig. 34). (X 100)**



	5 days	21 days
C	0.35	0.35
carr	0.85	0.68
AFA	0.34	0.37
AFA+Carr	0.38	0.36

Fig. 35:mean values of PAS positive materials relative to the control value.

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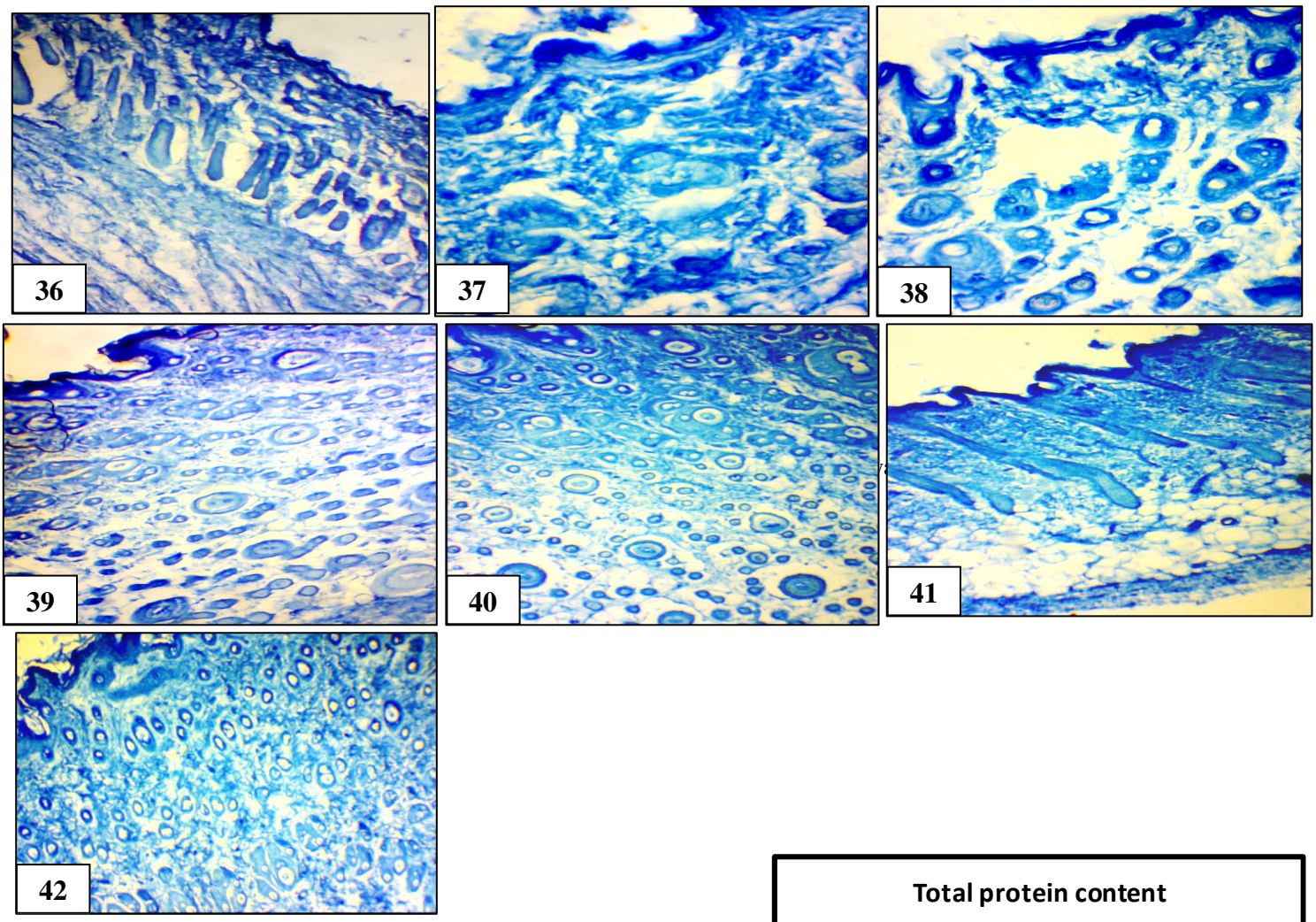

Figs. 36-42: photomicrographs of sections in skin tissue of the control and treated groups. (Mercuric bromophenol blue)

Fig. 36: photomicrograph of a section of the skin of the control group showing normal protein content in the epidermal and dermal layers.**Notice:** moderately stained epidermal layers, hair follicles and sub-dermal muscle fibres with less stained connective tissue. **(X 100)**

Figs. 37, 38:photomicrographs showing densely stained total protein all over the skin tissue of carrageenan group after five **(Fig. 37)** and twenty one days **(Fig. 38)**. **(X 200)**

Figs. 39, 40:photomicrographs of sections of the skin of AFA groupshowing normal distribution of protein content all over the skin tissue after five (**Fig. 39** and twenty one days **(Fig. 40)**. **(X 100)**

Figs. 41, 42:photomicrographs showing slightly increased total protein content in the epidermal and dermal layers in the skin tissue of AFA+ Carr groups compared to the control group after five **(Fig. 41)** and twenty one days **(Fig. 42).** **(X 100)**

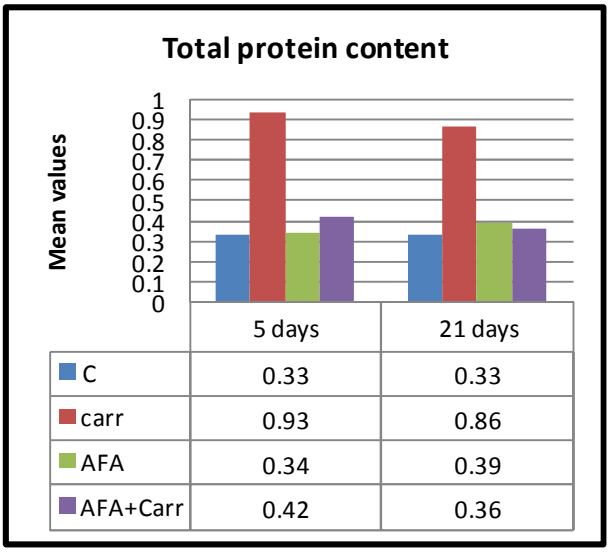

Fig. 43: mean values of total protein content relative to the control value.

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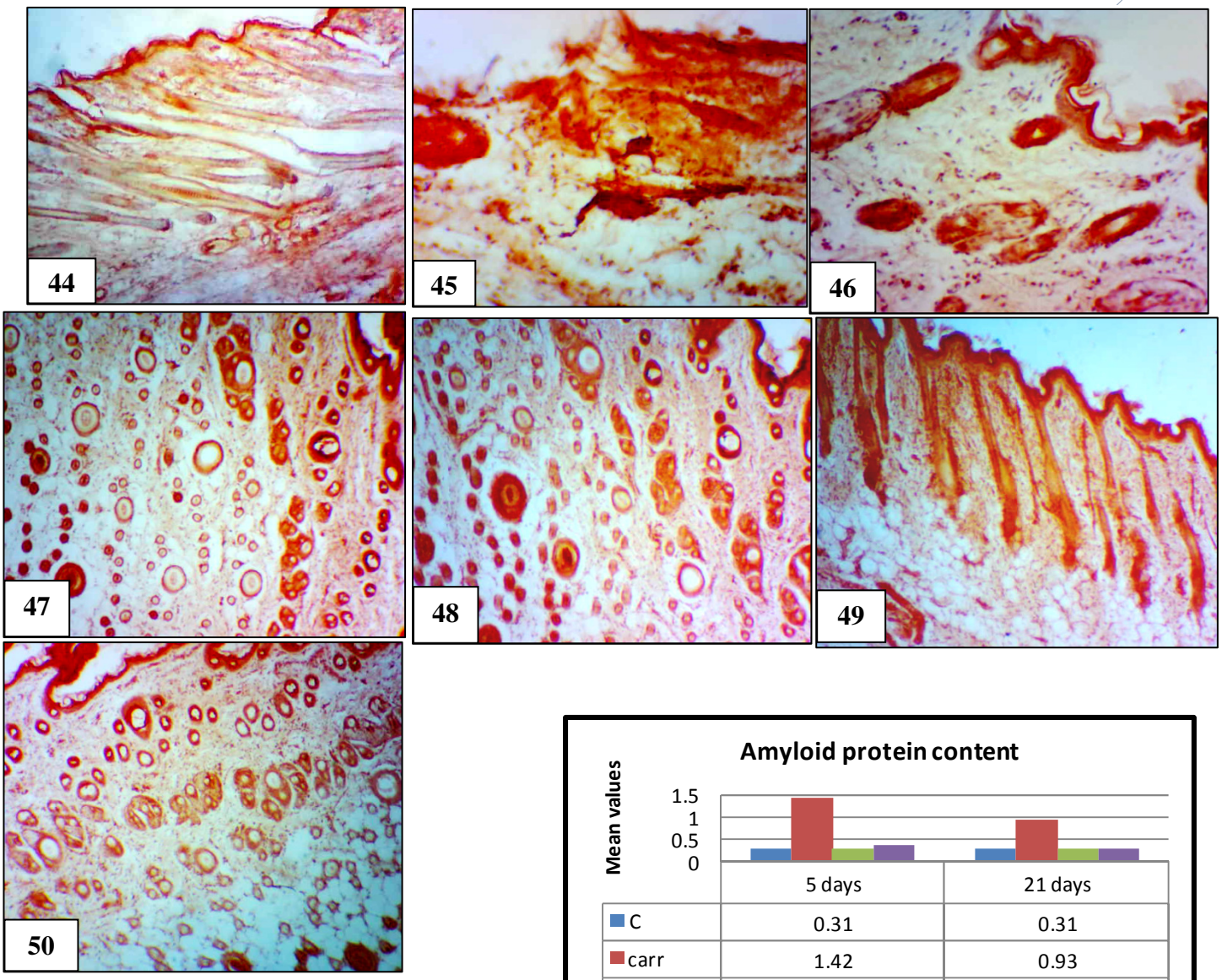

Figs. 44-50: photomicrographs of sections in skin tissue of the control and treated groups. (Congo red X 100)

Fig. 44: photomicrograph of a section of the skin of the control group showing faintly stained amyloid protein in the dermal and epidermal layers.

Figs. 45, 46:photomicrographs showing increased amyloid protein in the corrugated epidermal and destructed dermal layers of the skin tissue of carrageenan treated group after five **(Fig. 45)** and twenty one days **(Fig. 46)**.

Figs. 47,48:photomicrographs of sections of the skin tissue of groupAFA showing somewhat normal distribution of amyloid protein in the epidermal and dermal layers after five **(Fig. 47)** and twenty one days **(Fig. 48)**.

Figs. 49, 50:photomicrographs showing slightly increased amyloid protein content in the epidermal and dermal layers in the skin tissue of AFA+ Carr group after five days **(Fig. 49)** and normal distribution after twenty one days **(Fig. 50)** as compared to the control group**.**

Fig.(51): Mean values of amyloid protein content relative to the control value**.**

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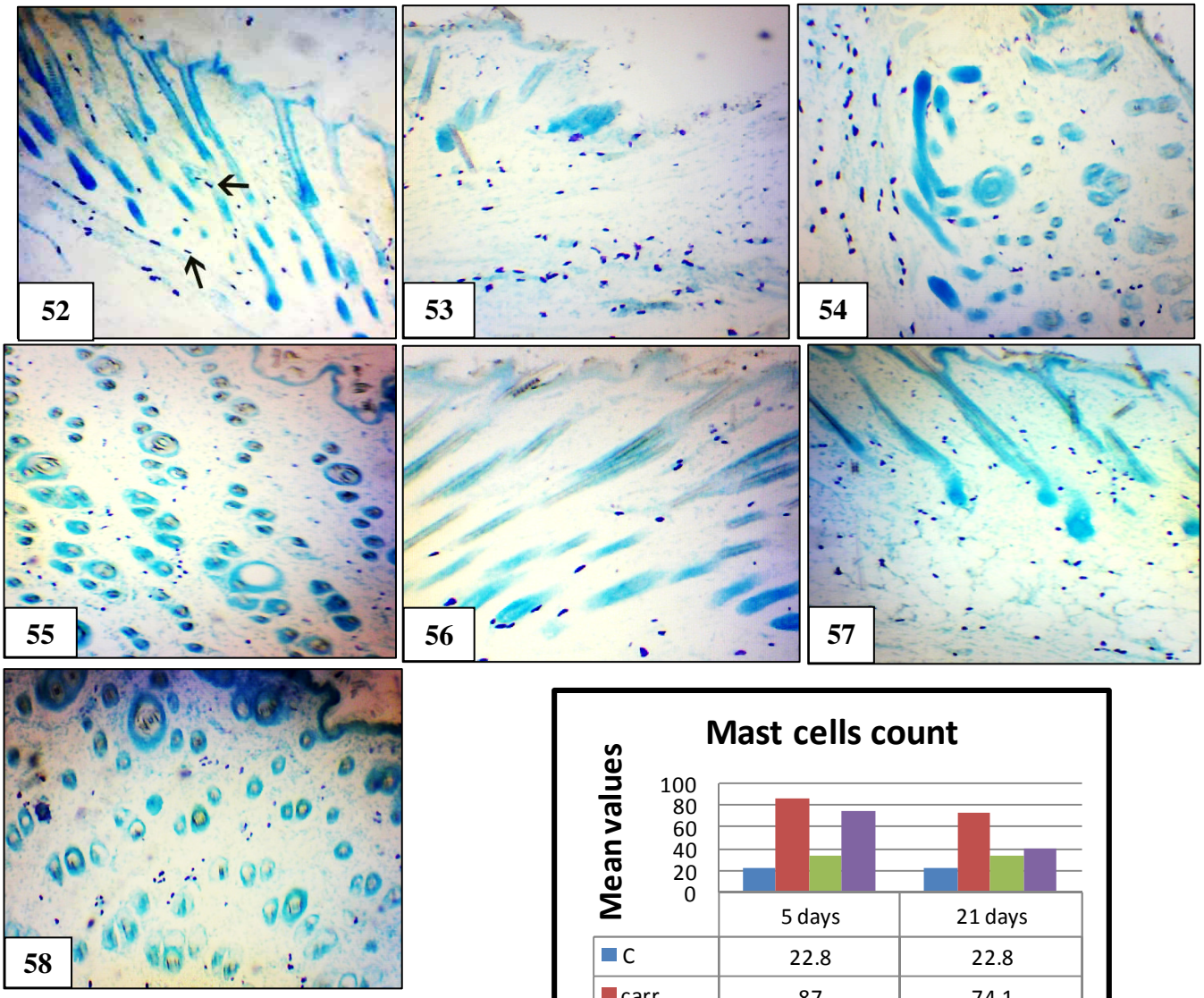

Figs. 57-58: photomicrographs of sections in skin tissue of the control and treated groups. (Toluidine blue X 100)

Fig. 52: photomicrograph of a section of the skin of the control group showing normal distribution of mast cells (→).

Figs. 53,54:photomicrographs showing increased mast cells of carrageenan group after five **(Fig. 53)** and twenty on days **(Fig. 54)**.

Figs. 55,56:photomicrographs of sections of the skin of AFA group showing slightly increased mast cells after five **(Fig. 55)** and twenty one days **(Fig. 56).**

Figs.57,58:photomicrographs showing increased mast cells in the skin of AFA +Carr group after five **(Fig. 57)** and twenty one days **(Fig. 58)** post-treatment.

Fig. 59: mean values of mast cells relative to the control value.

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4. Discussion

Concerning the histopathological changes, in the present study, skin tissue of rats treated with carrageenan for five and21 days post-treatment resulted in several histopathological lesions including thickened keratin, distortion, fibrosis, cellular debris, loss of hair follicles, granuloma , loss of cellular structures. The present results come in agreement with the work of **Abdel-Raouf (2006)** who reported that skin of rats treated with carrageenan showed many degenerative changes included disturbed keratin layer and hair follicles, irregular epidermis, vacuolation in the dermis, increased fibroblasts, affected muscle fibers and small areas of hemorrhage. The chronic granuloma observed post-carrageenan injection was noticed by **Massimo (1972)** who stated that granuloma occurred mainly due to the marked cellular emigration which happened during the acute response.

Ezeamuzie and Njoku (1992),Houle *et al.* (2005) and Ma *et al.* (2013) reported that the histopathological results of edema paws showed that carrageenan-induced swelling and neutrophil accumulation at the site of inflammation**.**The accumulation of leukocytes at the inflammatory site results from the interaction between endothelial cells and leukocytes **(Granger and Kubes, 1994).**

The present results are supported by a work conducted by **Kumar *et al.* (2014)** who reported that the myofibrils were separated from the epidermis by a wide gap in the muscle tissue second day after the injection of carrageenan. Similarly, the muscle tissue at the site of injection exhibited leucocytic infiltration followed by necrotic myositis clearly indicating the property of carrageenan as an indicator of inflammation.

In the present study skin sections examined five and twenty one days following AFA administration showed nearly normal appearance of the epidermal and dermal layers, highly increased and well developed hair follicles with their sebaceous glands. Also, the epidermal and dermal layers of rats administrated AFA post-carrageenan injection were resuming their normal structure with highly increased and well developed hair follicles in the expanded dermal layer (after twenty one days).

Wound healing is a fundamental response to tissue injury that involves a complex set of cellular, physiological and molecular events targeted toward the restoration of the structural and functional integrity of the damaged tissue **(Priya *et al.*, 2004)**. Results of the current work are supported by the work done by **Hagino and Masanobu (2003)** who revealed that uses of algal proteins or their derivatives are important in conferring moisture retention on hair and skin. On the other hand, algal proteins show a strong affinity with hair or skin to improve their nourishments. Moreover, rich protein contents and biologically dynamic growth factors in many of the algae can facilitate the preparation of cosmeceuticals. Furthermore, cosmeceuticals are supposed to be involved in healing and repairing damaged skin with moisturizing and maintaining the nourishment as well.

Bhat and Madyastha (2000) revealed that mycosporine-like amino acids (MAAs), AFA-phycocyanins and AFA- phytochrome which, to different extents, are endowed by anti-inflammatory properties and powerful antioxidants.They also added that 5% to 10% of the dry weight of the algae is represented by phycocyanins molecules which have powerful anti-inflammatory effects, similar to those of non-steroidal anti-inflammatory drugs, but of course without any side effects. In particular, it has been proven that phycocyanins are selective COX-2 inhibitors, as powerful as the drugs rofecoxib and celecoxib. Phycocyanins also inhibit the formation of leukotriene B_4 (an inflammatory metabolite of arachidonic acid).

The present results also are in agreement with the those of **Patel *et al.* (2005) and Abd El-Baky *et al.* (2009)** who detected that proliferation and growth stimulation activities of blue green algae seem to be directly associated with either C-PC or potentially other unidentified compounds. They also added that blue green algae contain a mixture of proteins and carotenoids which interact synergistically in mediating proliferation of skin cells and hence contribute significantly to wound healing and tissue regeneration. Also, **Abed *et al.* (2009) and Plaza *et al.* (2009) reported** that the anti- inflammatory and antioxidants effect of blue green algae and C-PC satisfied the basic criteria for being used in wound management.

Kushak *et al.* (2000) reported that AFA decreased the plasma level of arachidonic acid, thus reinforcing its general anti-inflammatory activity. AFA is also rich in specific nutraceutical molecules, such as phenylethylamine (PEA) and unique AFA-phycocyanins, with strong antioxidant and anti-inflammatory properties on one hand and powerful neuromodulating and neuroprotective activities on the other hand **(Scoglio *et al.*, 2009).**

Gur *et al.* (2013) showed that firstly, phycocyanin found in blue green algae directly enhances wound repair by its anti-oxidant property and scavenging destructive free radicals mechanism. Secondly, stimulation of keratinocyte is one mechanism by which phycocyanin might enhance wound repair. They also added that the minerals, phycobiliproteins, vitamins, beta carotene, fatty acids, polysaccharides, phenolic compounds and volatile compounds present in the microalgae may accelerate wound healing by acting as an anti-oxidant and scavenging destructive free radicals responsible for cell death**.**

Ismail *et al.* (2013) showed that blue-green algae increase the stem cells trafficking or homing in animals

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through induction of a transient boosting in the population of stem cells in animal's circulatory systems. Two of these natural products are Stem Flo and Stem Enhance, which are extracted from AFA plant **(Jensen *et al.*, 2007). Mansilla *et al.* (2006)** supported that the mobilization, migration and differentiation of bone marrow stem cells in the target tissue constitute a natural phenomenon of healing in the human body. *Aphanizomenon flos- aquae* stimulates the release of Adult MSC which can facilitate quick healing of injured/fractured bone **(Ochube *et al.*,2017).**

The present results extend and support the work of **Vestola *et al.* (2014)** who revealed that BGA is high in various B vitamins (including B_{12}), carotenoids and minerals including calcium, iron, magnesium, selenium, manganese, potassium and zinc. It is also a great source of gamma-linoleic acid (GLA), an essential fatty acid that stimulates skin and hair growth. They also added that many of these vitamins and minerals have strong antioxidant properties which help to eliminate toxins and fight against diseases. **Yogianti *et al.* (2014)** recorded the inhibitory effects of dietary blue green algae on ultraviolet B (UVB)induced skin inflammatory responses and carcinogenesis, which were attributed to the algae's anti-inflammatory and antioxidant effects.

Maxim *et al.* (2015) showed that after the blue green algae powder had been orally administered for a period of eight weeks, a highly significant increase in the carotenoid concentration of skin was observed .They also added that oral administration of the powdered algae improved the antioxidant status of the skin as demonstrated by the highly significant increase of the carotenoid concentration. So, the obtained increase can be directly correlated with the increased ability of the skin to counteract generated free radicals.

As regards the collagen fibres in the present study highly increased collagen fibres and fibrotic areas were detected in the epidermal and dermal layers after 5 and 21 days post-carrageenan injection.

According to **Robertson and Schwertz (1953)**carrageenan injected subcutaneously in the guinea pigs was able to induce the development of a glaucomatous tissue containing large amounts of collagen. The glaucomatous tissue formation was maximal at about 1 week and was completely desorbed at 46 weeks **(Jackson, 1957)**. In the current study treatment of rats with AFA post-carrageenan injection showed increased collagen fibres in the dermal layer after five days, while somewhat normal distribution of collagen fibres was detected after twenty one days.**Maxim *et al.* (2015)**showed that the systemically administered blue green algae *(Spirulina platensis)* slightly but insignificantly improved the dermal collagen concentration. They expected that additional antioxidant protection of skin will result in a decrease the amount of free radicals interacting with the dermal elastic fibers, such as collagen and elastin.

In the present investigation carrageenan group exhibited a significant increase in PAS positive materials in the corrugated epidermal layer and destructed dermal layer all over the experimental periods.

In the current study AFA treated group and AFA + Carr group exhibited non-significant increase in PAS positive materials all over the experimental periods.

Seaweeds are found to be as a source of novel nutraceuticals sulfated polysaccharides and peptides**(Jimenez-Escrig *et al.*,2011).**Carbohydrates such as starch, sugars, glucose and other polysaccharide exist in microalgae**(Brown *et al.*, 1997).** There is no restriction for the use of dried total microalgae in foods or feeds, due to their high overall digestibility.Besides the biological functions of some microalgal species have been related to their polysaccharides. Complexes of polysaccharide from microalgae such as glucose and a variety of combinations of mannose, galactose, rhamnose, *N*-acetylglucosamide,*N*-acetylgalactosamine and arabinose; these complexes have immune stimulatory effects **(Pugh and Pasco, 2001)**

Concerning the histochemical changes of total protein the present results showed that carrageenan group exhibited a significant increase in total protein of skin tissue as compared to the control group all over the experimental periods.

Results of the present study come in agreement with the work done by **Jean *et al.* (2008)** who noticed that carrageenan up regulate pro-inflammatory proteins (inducible nitric oxide synthase (iNOS) and cyclooxygenase 2(COX- 2)) at the site of inflammation.

Results of the present study also come in agreement with those of **Vazquez *et al.* (2015)** who realized that there was diverse systemic changes including increased levels of acute phase proteins, such as C-reactive protein(CRP) and fibrinogen after carrageenan injection.

In the present study rats administrated AFA alone showed non-significant increase of total proteins in skin tissue after five and twenty one days as compared to the control value**.** While rats administrated AFA post-carrageenan injection exhibited a significant increase in protein content after five days and a non-significant increase after twenty one days as compared to the control value

The present results are supported by **Becker (2007)** who revealed that microalgae have the potential to become an alternative protein source due to the fact that several strains contain proteins of high quantities (more than 50% of dry weight) and qualities. The presence of essential amino acids, such as lysine, leucine, isoleucine and valine (comprising 35% of essential amino acids in muscle proteins of humans), contributes to the high quality of microalgal proteins, making them highly suitable for use as direct supplements (both for animal and human consumption) or as nutraceuticals**(Dewapriya and Kim, 2014).**

Spolaore *et al.* (2006) reported that an extract rich of protein from BGA *(Spirulina)* can repair the signs of skin aging and can stimulate collagen synthesis. **Kim and Wijesekara (2010)** revealed that algae bearing rich source

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of secondary metabolites, including functional nutrients and associated bioactive peptides, than other marine organisms. Indeed, algae bioactive peptides could be a natural source with prominent biological activities beside its health effects.

Concerning the histochemical changes of Amyloid-β protein the current study recorded a significant increase in amyloid β protein content in skin tissue of carrageenan group relative to the control group all over the experimental periods.

Amyloids are insoluble fibrous protein aggregates sharing specific structural traits. They are insoluble and arise from at least 18 inappropriately folded versions of proteins and polypeptides present naturally in the body(**Alvarado *et al*., 2000**). These misfolded structures alter their proper configuration such that they erroneously interact with one another or other cell components forming insoluble fibrils. They have been associated with the pathology of more than 20 serious human diseases in that abnormal accumulation of amyloid fibrils in organs may lead to amyloidosis and may play a role in various neurodegenerative disorders **(Pulawski *et al.*, 2012).**

Kadowaki *et al.* (2005) showed that amyloid deposition is associated with mitochondrial dysfunction and resulting generation of reactive oxygen species (ROS), which can initiate a signalling pathway leading to apoptosis.

The present study showed that rats administrated AFA alone and AFA post-carrageenan injection exhibited non-significant changes in amyloid β protein content relative to the control group after five and twenty one days post-treatment**.**

Studies have reported various health benefits of BGA, including immune functions, anti-inflammatory, anti-bacterial, anti-viral, anti-cancer, anti-diabitic, hypocholesterolemic and hypotriglyceridemic properties **(Jensen *et al*., 2008 ; El-Depsi, 2016).**The present study is supported by the work of **Yang *et al*. (2011)** who revealed that long-term BGA supplementation in mice did not induce any evident adverse side-effects.

Concerning the histochemical changes of mast cells count the present study showed a significant increase in mast cells count in skin tissue of carrageenan treated group after five and twenty one days post-treatment.

Vinegar *et al.* (1987) observed that dermal mast cells increased in injured skin by carrageenan injection.

The present investigation comes in agreement with the result of **Radhakrishnan *et al*. (2003)** who reported that inflammatory changes was observed histologically for both the joint and muscle tissues after injection of carrageenan. Acute inflammation was observed for the first 24 h with edema and neutrophilic infiltration evident as early as 4 h. At 1 week, the inflammation converted to primarily macrophage response with scattered mast cells.

Mast cells are an important source of cytokines during inflammatory responses. In various settings, they have the capacity to release a wide array of cytokines **(Kalesnikoff and Galli, 2008).** Mast cells also facilitate the interaction of the central circulatory system with a local inflammatory site, in part by promoting the recruitment of cells into a site of inflammation, primarily from the bloodstream. For example, mast cells can mediate early neutrophil recruitment, which is often the first line of defense against pathogens (**St-John *et al*., 2011).** Because of their preponderance at the host-environment interface and their large repertoire of cell surface receptors, such as the high-affinity IgE receptors and complement component receptors, mast cells are capable of responding to a wide variety of exogenous and endogenous stimuli, making them versatile detectors of allergens, tissue injury and infection **(Abraham and St John, 2010).**

In the present study AFA treated group exhibited non-significant increase of mast cells in the dermal layer all over the experimental periods, while rats administrated AFA post-carrageenan injection exhibited a significant increase of mast cells after five days with non-significant increase after twenty one days.

Romy *et al*. (2003) reported that phycocyanin reduced edema, histamine (Hi) release, myeloperoxidase (MPO) activity, the levels of prostaglandin (PGE2) and leukotriene (LTB4) in the inflamed tissues. These anti-inflammatory effects of phycocyanin can be due to its scavenging properties toward oxygen reactive species (ROS), its inhibitory effects on COX-2 activity and on Hi release from mast cells**.**

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