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RESEARCH ARTICLE

Protective and therapeutic effects of fucoidan, brown algae extract, against methotrexate hepatic toxicosis in albino rats

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Abstract

Fucoidan has been shown to have anti-inflammatory, antioxidant and promoted the immune function. Additionally, fucoidan also has been reported to reduce liver fibrogenesis by protecting from hepatic cell death and inducing hepatic stellate cells (HSC) death. The present study used fifty Swiss male albino rats (150-175 g) to assess the protective and therapeutic effects of fucoidan, brown algae extract, against oxidative stress on the hepatic damage in liver of the experimental rats treated with methotrexate (MTX). The rats were equally divided randomly into five groups. Elevated serum enzyme, hepatic necrosis, fibrosis, were noticed in rats intramuscularly (IM) injected with MTX (3 mg/ kg B wt). Fucoidan administration (200 mg/kg B wt) prior (protective group) and simultaneously with MTX ameliorated the induced hepatic damage. It lowered the serum enzymes and malondialdehyde (MDA) formation besides restoring the normal histological structure. Fucoidan (therapeutic trials) slightly ameliorated the effects previously induced by MTX when compared with the protective trial. Suppressive effect of fucoidan on activated hepatic stellate cells (HSC) was evaluated using immunohistochemical staining. In conclusion, our finding proved that fucoidan administration prior and simultaneous or after MTX induced protective and therapeutic effects. Moreover, it reduced the hepatic lesions through scavenging oxidative stressors and down regulating the pro-inflammatory cytokines.

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INTRODUCTION

Rheumatoid arthritis is the most common inflammatory autoimmune disorder, causing progressive joint destruction as a result of chronic synovitis **(Puszczewicz and Iwaszkiewicz 2011, Eberhardt, 2007)**. MTX, in low dose, is used commonly as a cytotoxic agent in the treatment of leukemia and other malignancies as well as in the inflammatory diseases such as psoriasis and rheumatoid arthritis. Efficacy and toxicity for MTX appear related to absorbed dose of MTX, not to route of administration **(Goodman et al., 2014, Ghaffar et al., 2011, Sener et al., 2006[a], Sener et al., 2006[b])**. Even at low doses, MTX has been associated with hepatic disorders, mostly elevated liver enzymes, that may lead to treatment cessation **(Visser et al., 2009, Kremer et al., 1994)** The histological abnormalities in patient treated with MTX include non specific histological feature such as fatty change, focal liver cell necrosis, portal tract inflammation, nuclear pleiomorphism and fibrosis with collagen accumulation in the peri-sinusoidal space **(Laharie et al., 2008, Gilbert et al., 1990).** With the widespread use of MTX, hepatotoxicity seems to be the most important potential major side effect **(West, 1997).** The pathogenesis of drug or toxin induced liver injury usually involves the toxic metabolites that either elicit an immune response or directly affect the biochemistry of the cell. Intracellular stress can lead to apoptotic or necrotic cell death, depending on the extent of mitochondrial involvement and the balance of factors that activate and inhibit the Bcl 2 family of proteins and the caspases. Nowadays, it is well known that drug metabolites promote a variety of chemical reactions including depletion of reduced glutathione (GSH) or

promotes oxidative stress with consequent effects on proteins, lipids and DNA resulted in mitochondrial and nuclear damage **(Uraz et al., 2008, Kaplowitz, 2002, Gill, 2001).** However, the number of laboratory animal studies in which microscopic and biochemical changes have been evaluated together are scarce.

Brown seaweeds have been the mainstay of the Japanese diet and have been documented in traditional Chinese medicine for over 1000 years **(McLellan, 1992).** Fucoidan is a sulfated polysaccharide purified from brown algae, such as Fucus vesiculosus, and has been shown to have various pharmacological properties such as hematopoietic progenitor cells activation **(Choi et al., 2005, Sweeney et al., 2002, Frenette et al., 2000),** anticoagulant **(Matsubara et al., 2001),** antitumor **(Maruyama et al., 2003)**, anti-inflammatory **(Angstwurm et al., 1995)**, antioxidant **(Maka et al., 2013, Hu et al., 2003)** and promoted the immune function (stimulates multiple functions of dendritic cells) **(Kim et al., 2008, Yang et al., 2003).** Fucoidan also protected tissues from various types of injury by increasing the hepatic growth factor **(Fukuta and Nakamura, 2008).** Fucoidan has recently been reported to reduce liver fibrogenesis by protecting from hepatic cell death and inducing HSC death **(Hayashi et al., 2008)** as well as showing anti-oxidative properties against CCl4-induced acute liver injury **(Kang et al., 2008)**. However, the mechanism by which fucoidan elicits its hepatoprotective effect on chronic liver fibrosis has previously been reported **(Hong et al., 2012).**

This experimental study was conducted to assess the possible protective and therapeutic effects of fucoidan, brown algae extract, to alleviate the pathological changes in liver of the experimental rats treated with MTX.

MATERIALS AND METHODS

Experimental animals and design:

Fifty Swiss male albino rats (150-175 g) were divided randomly into 5 equal groups which separately housed in five clear polycarbonate cages. They were provided with a standard animal diet and water ad libitum. The rats were kept at a temperature of 21 ± $2C^{O}$ in a 12-hour light/dark cycle. All experiments were performed in accordance with protocols approved by the Animal Care and Use Committee of Najran University (Saudi Arabia kingdom). Gp 1 (control) was received the equivalent volumes of saline. Gp 2 was orally given fucoidan (200 mg/kg B wt) for 90 days **(Raghavendran et al., 2007)**. Gp 3 was IM injected with MTX (3 mg/ kg B wt) twice weekly for 45 days **(Lin et al., 2014).** Gp 4 (protective) was daily orally given fucoidan (200 mg/kg B wt) for 45 days. After that, the rats were IM injected with methotrexate (3mg /kg B wt) twice weekly for another 45 days, 2 hours after fucoidan treatment. Gp 5 (therapeutic) was IM injected with methotrexate (3 mg/kg B wt) twice weekly for 45 days and then fucoidan (200 mg/kg. B wt) was orally given for other 45 successive days.

Chemicals:

Methotrexate was purchased from David Bull Laboratories, Mulgrave – Victorica, Australia. Fucoidan powder was purchased from Sigma Chemical, Co. Ltd. (St. Louis, MO, USA). The chemicals were stored under proper conditions according to Sigma instructions. All other chemicals used will be of the finest analytical grade.

Assay of serum enzymes

At the end of the experiment, blood was obtained from the tail vein. Serum was separated and was used for analysis of the following indices: alanine aminotransferase (ALT), aspartate aminotransferase (AST) was evaluated by using commercial test kits (Randox Laboratories LTD Co., UK.) according to **(Reitman, 1957)**, GSH was evaluated by using readymade kits provided by Randox Laboratories LTD Co. UK. Lipid peroxide malondialdehyde (MDA) was also determined by enzymatic colorimetric method by using readymade kits provided by Biodiagnostic according to **(Satoh, 1978)**. Creatinine and urea were evaluated by using kits provided by Colorimetric Randox, UK. MDA (Bioxytech Oxis International, Inc) according to **Satoh K 1978**.

Pathological Examinations

All animals were humanely sacrificed at the end of experiment. Liver specimens were collected for histopathological examination. Specimens were immediately fixed in neutral buffered formalin and 5µ thick paraffin sections were prepared, stained with hematoxylin and eosin (**Bancroft et al., 1990)**. The stained sections were examined blindly by two experts for evaluation of hepatic lesions.

Immunohistochemical study of αSMA

For the detection of αSMA, a rat monoclonal primary antibody against αSMA (Sigma, Munich, Germany) was used. Antigen localization was achieved using the avidin-biotin complex (ABC) technique **(Hsu et al., 1981)**.

Statistics

Data analysis was achieved using the Graph- Pad Instat software (Version 2.0 Philadelphia, 1993). Data were expressed as mean ± SD. Comparisons were done using one-way ANOVA followed by Tukey-Kramer as post ANOVA test. Criterion for significance was chosen to be at $p \leq 0.05$.

RESULTS

Assessment of hepatic function using biochemical tests

Table (1) showed that fucoidan significantly decreased the serum transaminases in gps 4 & 5 in comparison with gp 3. Moreover, it markedly increased the GSH in gps 4 & 5 than gp 3, where it significantly decreased when compared

with the control. Furthermore, gp 4 showed marked increase in GSH than gp 5. The MDA was significantly increased in gp 3 when compared with the control; meanwhile, it was significantly decreased in gps 4& 5, than gp 3 and was decreased in gp 4 than gp 5.

Pathological finding

Hematoxylin and Eosin stain:

The liver of control rats showed a normal histological appearance of hepatocyte and sinusoidal architectures (Fig 1). However, in fucoidan-treated rats showed slight periportal increase in hepatocytes eosinophilia (Fig 2), distended sinusoid with neutrophilic band cells of extrahematopoiesis (Fig 3) and slight activation of the kupffer cells (Fig 4). In the MTX-treated rats, the liver showed extensive coagulative necrosis invaded or completely replaced with lymphocytes and eosinophils (Figs 5 and 6). Periportal areas of fibrous connective tissue proliferation infiltrated with numerous macrophages engulfed yellowish brown hemosiderin pigments were visualized (Figs 7 and 8). Organized thrombus and foamy hepatocytes and macrophages were noticed in the fibrotic areas (Fig 9). Congestion and hemorrhage were detected throughout the hepatic parenchyma (Fig 10). Micro and macro-vesicular steatosis were seen (Fig 11). Numerous megalohepatocytes and diploid hepatocytes with enlarged nuclei were detected (Fig 12). The normal radial arrangements of hepatocytes from central vein were severely distorted with severe cholestasis. The latter was represented by yellowish or brown pigment among the hepatocytes. Foci of apoptotic cells were detected. In rats of protective group showed significant reduction in the lesions that induced by MTX alone or individually absent and restored to the normal appearance. The reported lesions were represented by swollen and mild hydropic degeneration in the periportal hepatocytes (Fig 13). Hypertrophy and activation of the kupffer cells (Fig 14) were seen with multiple aggregates of neutrophilic band cells and erythroid precursor in the sinusoids and congestion (Fig 15). Sometimes, the portal areas revealed congested portal vein and round cells infiltration (Fig 16). Apoptotic cells were not observed in this group. However, fucoidan treatment did not completely alleviate the lesions, and severe congestion (Fig 17), small areas of coagulative necrosis infiltrated with macrophages and eosinophils (Fig 18) and intense periacinar aggregations of round cells and eosinophils (Fig 19). The portal areas revealed biliary and oval cells proliferation besides few fibrous connective tissues infiltrated with round cells and eosinophils (Fig 20).

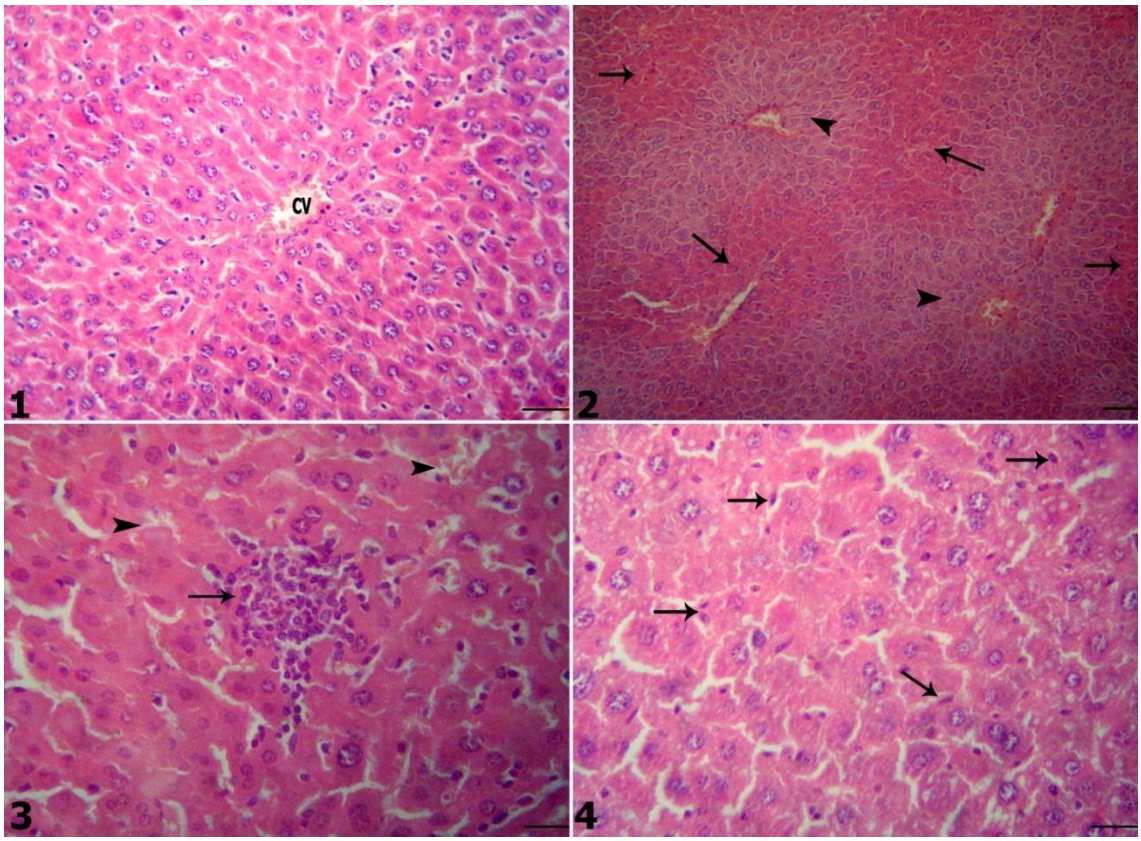
Effect of fucoidan on α-SMA expression in hepatic stellate cells

The data of immunohistochemical stained sections of livers were given in Figs (21-26). A normal liver or that only received fucoidan showed normal expression of α-SMA positively stained brown HSC (Figs 21 and 22). The Immunohistochemical stained sections of liver treated with MTX showed strong immunoreactive expression of α-SMA represented by brown color of α- SMA positively among hepatic stellate cells mainly around center veins and forming intra-acinar thick bands (Figs 23 and 24). In contrast, the protective group showed little brown coloration scattered around hepatic sinusoids (Fig 25). The treated-group showed deceased the number of positive stained cells comparable with the MTX (Fig 26).

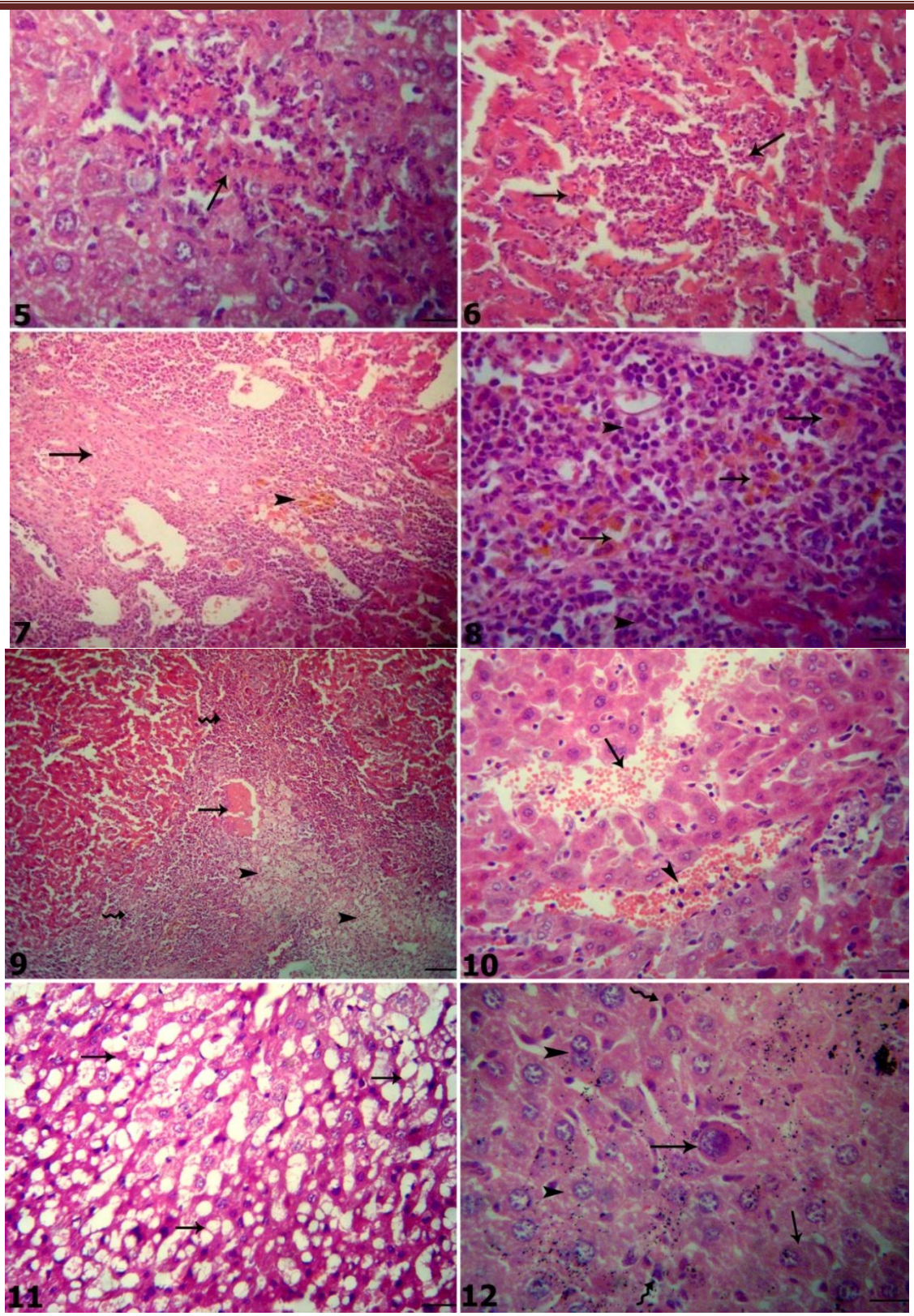
Table (1): Mean enzyme activity and concentration with standard error

	AST	ALT	GSH	MDA
Group I	23.45 ^{a} ±2.51	19.58 ^{a} ±1.20	2.27 ^{a}±0.23	0.295 ^{a}±0.023
Group II	23.55 ^{a} ±2.90	20.52 ^{a} ±1.04	2.85 ^{a} ±0.15	0.289 ^{a} ±0.016
Group III	60.34 ^{c} ±4.8	58.15 ^{c} ±3.36	0.92 ^{c} ±0.09	1.60 ^{c}±0.19
Group IV	35. ^{b} ±3.17	32.75 ^{b} ±1.66	1.65^{b} ±0.12	0.55 ^{b} ±0.021
Group V	44.5 ^{b} ±2.10	41.56 ^{b} ±2.42	1.32 ^{b} ±0.15	0.81 ^{b} ±0.04

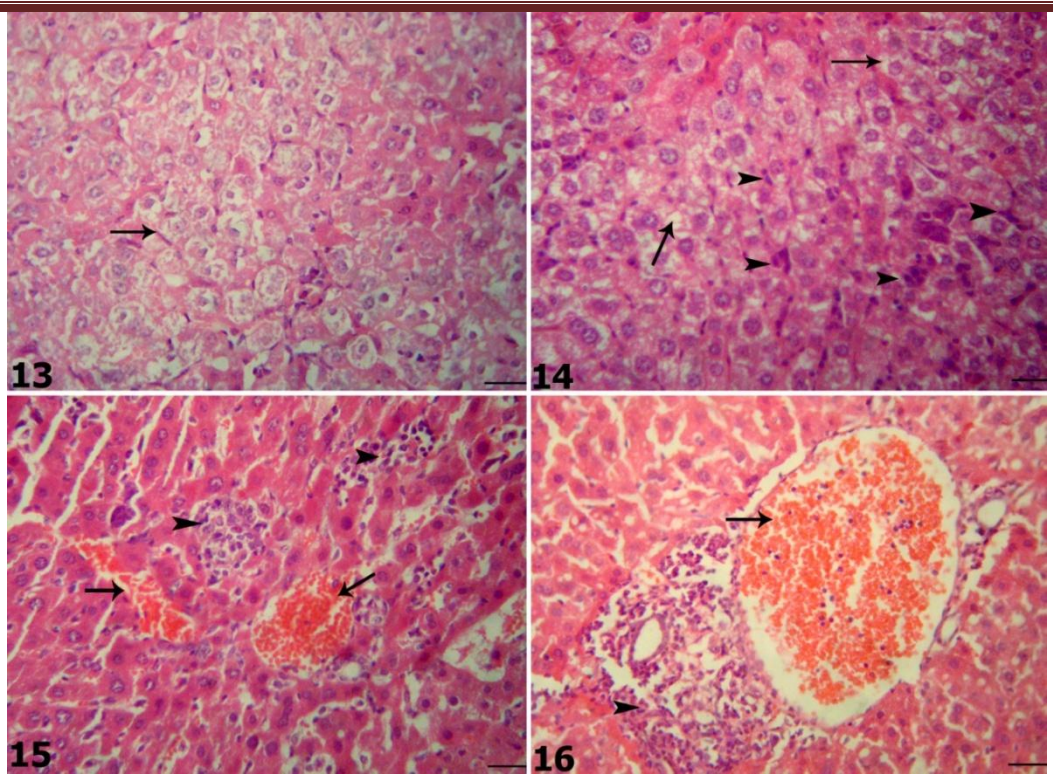
Mean with the same letter is showing a non-significant change
Mean with different letters is showing a significant change



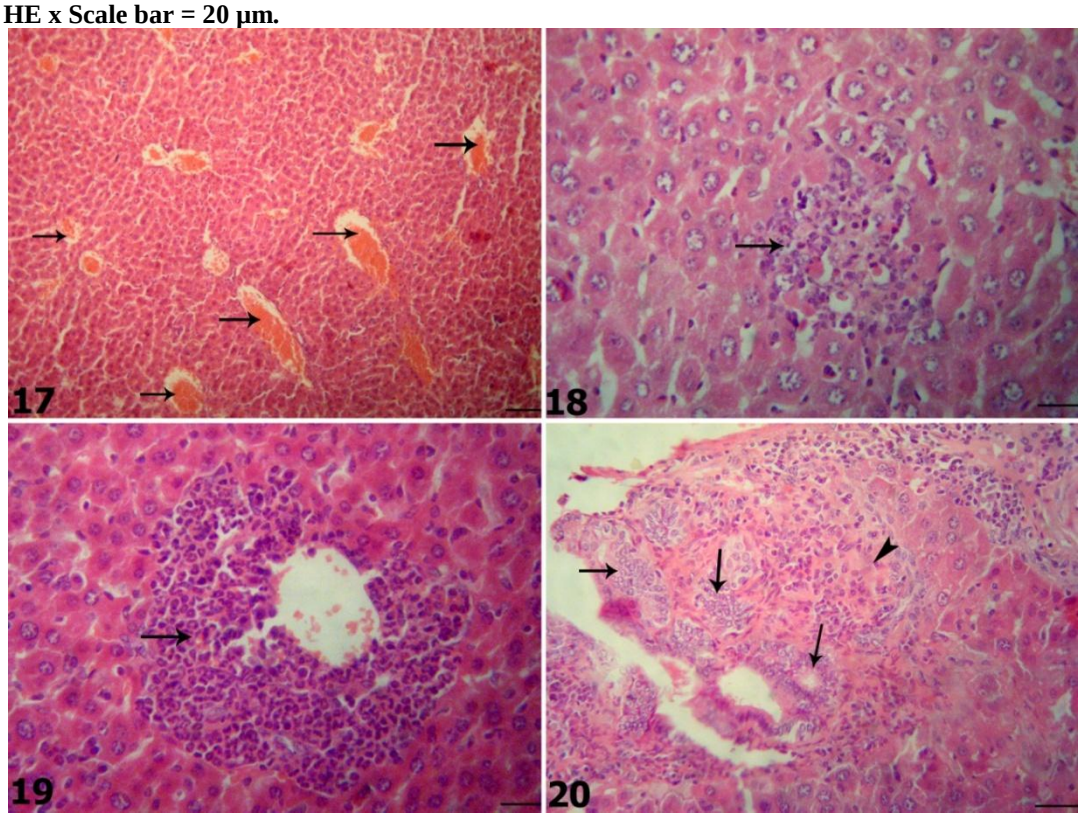
Figs (1-4): Liver of control or received Fucoidan rat shows: normal hepatocytes and sinusoidal architectures in the control (1) and in the Fucoidan periportal increase in hepatocytes eosinophilia (2), distended sinusoid with neutrophilic band cells of extrahematopoiesis (3) and activation of the kupffer cells (4). **HE x Scale bar = 20 µm.**



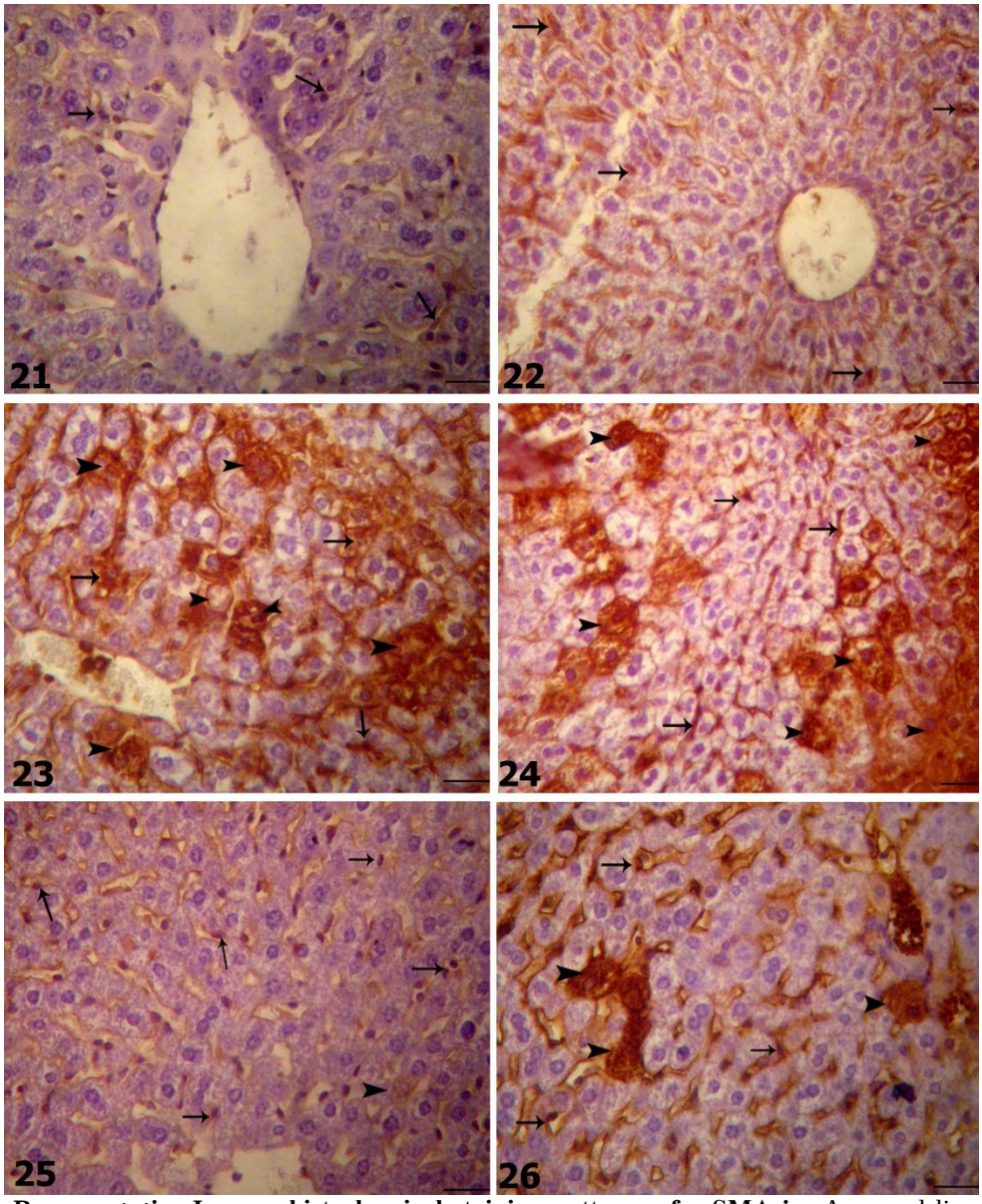
Figs (5-12): Liver of rat received MTX shows: extensive coagulative necrosis invaded (5) or completely replaced with lymphocytes and eosinophils (6), area of fibrous connective tissue proliferation (7) infiltrated with numerous macrophages engulfed willowish brown hemosiderin pigments (8), organized thrombus and foamy hepatocytes and macrophages in the fibrotic area (9), congestion and hemorrhage (10), macrovesicular steatosis (11) and megalohepatocytes and neumerous diploid hepatocytes (12). **HE x Scale bar = 20 µm.**



Figs (13-16): Liver of rat of protective group shows: swollen and mild hydropic degeneration (13), hypertrophy and activation of the kupffer cells (14), multiple aggregates of neutrophilic band cells and erythroid precursor in the sinusoids and congestion (15) and portal area with congested portal vein and round cells infiltration (16). **HE x Scale bar = 20 µm.**



Figs (17-20): Liver of rat of therapeutic group shows: severe congestion (17), small area of coagulative necrosis infiltrated with macrophages and eosinophils (18), intense periacinar aggregation of round cells and eosinophils (19) and portal area with biliary and oval cells proliferation besides few fibrous connective tissue infiltrated with round cells and eosinophils (20). **HE x Scale bar = 20 µm.**



Figs (21-26): Representative Immunohistochemical staining patterns of α SMA in: A normal liver or that only received fucoidan shows normal expression of α- SMA for hepatic stellate cells (21, 22), Strong immunoreactive expression of α- SMA represented by brown color of α- SMA positively myofibroblasts among hepatic stellate cells with MTX. Notice the intra-acinar reaction of the delicate fibrous proliferation (23, 24), Weak immunoreactive response of α- SMA of hepatic stellate cells in protective group (25) and moderate immunoreactive staining in the therapeutic in compared with MTX (26). **Scale bar = 20 µm.**

DISCUSSION

The current investigation showed that fucoidan protected and partially ameliorated the adverse effects of MTX-injury and fibrogenesis, in rat liver, by scavenging the oxidative stress suppressing the hepatic inflammation, attenuating the hepatic oxidative stress, and inhibiting the HSC-activation. The rat was proven to be an ideal biomonitor for hepatic fibrosis **(Semiha et al., 2006).** Drugs used for cancer chemotherapy are well known to produce acute toxic side effects in multiple organ systems **(Kim, 1999).** It has been reported that liver damage may occur as well in particular high doses or following chronic administration of MTX **(Uraz et al., 2008, Sener et al., 2006[a]).** MTX causes oxidative tissue damage by increasing lipid peroxidation in the liver tissue and decreasing the level of antioxidant enzymes, which cause hepatic necrosis, inflammation, and fibrogenesis. The conversion of MTX to its major extracellular metabolite (7-hydroxymethotrexate) takes place in the liver, where it is oxidized by a soluble enzymatic system.

(Johovic et al., 2003). Moreover, increased AST and ALT values, biochemical indicators of liver damage, and histopathological findings supported this conclusion **(Vardi et al., 2010).** The current study revealed that fucoidan is a potent antioxidant through a marked decrease in MDA and increase in GSH in gps 4&5 when compared with gp 3. Moreover, ALT and AST activities were decreased in gps 4&5 when compared with gp 3. The histopathology of our study revealed improved hepatic architecture in both protective and therapeutic groups than MTX treated group. Chronic hepatitis is commonly associated with fibrogenesis **(Marra et al., 2002)**. Von Kupffer cells are the resident hepatic-macrophages, which upon activation release toxic cytokines and ROS which kills the hepatocytes and promotes inflammatory responses **(Luckey & Petersen, 2001).** The Kupffer cells are activated through the TLR4 expressed on their cell-surface those results in nuclear factor- kappa beta (NF-k β) and activator protein-1 (AP-1) activation. The NF-k β is translocated into the nucleus to activate the transcriptional factors, of proinflammatory IL-1, IL-6 and TNF-α that mediate the inflammatory response **(McGavin & Zachary, 2003).** Quite unexpectedly, the hepatic stellate cells appeared to be involved in the hepatic immune response, since they expressed TLRs **(Notas et al., 2009)**. NF- k β is anti-apoptotic for HSC and ensures that these cells persist despite exposure to a variety of pro-apoptotic stimuli **(Lang et al., 2000).** In our study the improved pathological and immunohistochemical picture of liver in gps 4&5 than gp 3 could be attributed to inhibition of proinflammatory cytokines expression that suppressed inflammation and increased sensitivity of HSC to apoptosis and subsequently this can lead to a compensatory hepatocyte- proliferation. This was confirmed by the increased regenerative capacity of hepatocytes in gps 4&5.

Liver fibrosis is a consequence of chronic hepatitis and involves the abnormal accumulation of extracellular matrix proteins, particularly collagen **(Tsukada et al., 2006).** The hepatic stellate cells (HSC) account for 5–8% of the total cell-count in the normal liver. They are located in the perisinusoidal spaces of Disse, in between the sinusoidal endothelium and hepatocytes, with a higher frequency in the periportal areas than centrolobular **(Wake, 1995).**The HSC are the primary cells, in the liver, that are responsible for excess collagen synthesis during hepatic fibrosis **(Zakim et al., 2003).** The activation of the quiescent HSC and their subsequent differentiation into myofibroblasts-like cells is very reliably indicated by the expression of α-smooth muscle actin, an actin isoform which is absent in the other resident liver cells in both normal and injured liver **(Rubbia-Brandt et al., 1997).** Among the large variety of cytokines, secreted by HSC, is TGF-β1 which most likely represents the highest impact on collagen over-production and accumulation in liver fibrosis **(Poli, 2000).** The examination of immunohistochemical stained tissue in our work confirm that fucoidan reduced the MTX-induced liver fibrosis. The immunohistochemical stained sections of liver treated with MTX showed strong immunoreactive expression of α- SMA. In contrast, the protective and therapeutic groups showed little brown coloration scattered around hepatic sinusoids. Low grade of fibrosis was encountered in gps 4&5, while, high grade of fibrosis were recognized in gp 3. It could be concluded that the oral administration fucoidan (prophylactic) was highly effective in preventing and reversing the MTX-induced hepatic injury and fibrosis. Meanwhile the treatment trials showed moderate improvement of hepatic function parameters. Such beneficial action could block of the oxidative stress and suppressing pro-inflammatory cytokines and subsequently inflammatory cell infiltration.

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REFRENCES

1. Angstwurm K, Weber JR, Segert A, Bürger W, Weih M, Freyer D, Einhäupl KM and Dirnagl U (1995): Fucoidan, a polysaccharide inhibiting leukocyte rolling, attenuates inflammatory responses in experimental pneumococcal meningitis in rats. Neurosci Lett, 19: 191:1-4.

2. Bancroft J, Stevens A and Turner D (1990): Theory and practice of histological technique 3rd Ed., Churchill, Livingstone, Edinburgh, London, Melbourne Publishers.

3. Choi EM, Kim AJ, Kim YO and Hwang JK (2005): Immunomodulating activity of arabinogalactan and fucoidan in vitro. J Med Food, 8: 446-453.

4. Eberhardt K, Larsson BM, Nived K, Lindqvist E (2007): Work disability in rheumatoid arthritis development over 15 years and evaluation of predictive factors over time. J Rheumatol, 34 (3): 481-7.

5. Frenette PS and Weiss L (2000): Sulfated glycans induce rapid hematopoietic progenitor cell mobilization: evidence for selectin-dependent and independent mechanisms. Blood, 96: 2460-2468.

6. Fukuta K and Nakamura T (2008): Induction of hepatocyte growth factor by fucoidan and fucoidan-derived oligosaccharides. J Pharm Pharmacol, 60 (4): 499-503.

7. Ghaffari AR, Noshad H, Ostadi A, Ghojazadeh M, Asadi P (2011): The effects of milk thistle on hepatic fibrosis due to methotrexate in rat. Hepat Mon, 11(6): 464-8.

8. Gilbert SC, Klintmalm G, Menter A, Silverman A (1990): Methotrexate-induced cirrhosis requiring liver transplantation in three patients with psoriasis. A word of caution in light of the expanding use of this 'steroid-sparing' agent. Arch Intern Med, 150: 889–91.

9. Gill RQ, Sterling RK (2001): Acute liver failure. J. Clin. Gastroenterol, 33 (3): 191-8.

10. Goodman SM, Cronstein BN, Bykerk VP (2014): Outcomes related to methotrexate dose and route of administration in patients with rheumatoid arthritis: a systematic literature review. Clin Exp Rheumatol., Dec 23. [Epub ahead of print]

11. Hayashi S, Itoh A, Isoda K, Kondoh M, Kawase M and Yagi K (2008): Fucoidan partly prevents CCl4-induced liver fibrosis. Eur J Pharmacol, 580 (3): 380-394.

12. Hong S, Lee H, Jung K, Lee H, Hong S (2012): Protective effect of fucoidan against acetaminophen-induced liver injury. Arch Pharm Res, 35(6): 1099-105.

13. Hsu SM, Raine, L, and Fanger H (1981): A Comparative study of the peroxidase-antiperoxidase method and an avidin-biotin complex method. Am.J. Clin. Pathol., 75: 734.

14. Hu T, Liu D, Chen Y, Wu J and Wang S (2003): Antioxidant activity of sulfated polysaccharide fractions extracted from Undaria pinnitafida in vitro. International Journal of Biological Macromolecules, 46: 193–198.

15. Johovic N, Cevik H, Sehirli O, Yegen B and Sener G (2003). Melatonin prevent methotrexate- induced hepatorenal oxidative injury in rats. Journal of Pineal Research, 34: 282–7.

16. Kang K, Kim ID, Kwon R, Lee J, Kang JS and Ha BJ (2008): The effects of fucoidan extracts on CCl4-induced liver injury. Arch Pharm Res, 31 (5): 622-627.

17. Kaplowitz N (). Biochemical and cellular mechanisms of toxic liver injury. Semin Liver Dis 2002, 22 (2): 137-44.

18. Kim, JC, Kim, KH and Chung, MK (1999): Testicular cytotoxicity of DA-125, a new anthracycline anticancer agent, in rats. Report Toxicol, 13: 391–7.

19. Kim MH and Joo HG (2008): Immunostimulatory effects of fucoidan on bone marrow-derived dendritic cells. Immunol Lett, 115, 138-143.

20. Kremer JM, Alarcon GS, Lightfoot RW JR (1994): Methotrexate for rheumatoid arthritis. Suggested guidelines for monitoring liver toxicity. American College of Rheumatology. Arthritis Rheum, 37: 316–28.

21. Laharie D, Terrebonne E, Vergniol J, Chanteloup E, Chabrun E, Couzigou P, de Lédinghen V (2008): The liver and methotrexate. Gastroenterol Clin Biol, 32 (2): 134-42.

22. Lang A, Schoonhoven R, Tuvia S, Brenner DA and Rippe RA (2000): Nuclear factor kappa β in proliferation, activation, and apoptosis in rat hepatic stellate cells. J. Hepatol, 33: 49–58.

23. Lin B, Zahao Y, Han P, Yue W, Ma XQ, Rahman K and Han T (2014): Anti-arthritic activity of Xanthium strumarium L. extract on complete Freund′s adjuvant induced arthritis in rats. J Ethnopharmacol, 8, 155 (1): 248-55.

24. Luckey SW and Petersen DR (2001): Activation of Kupffer cells during the course of carbon tetrachloride-induced liver injury and fibrosis in rats. Exp Mol Pathol, 71: 226-40.

25. Maka W, Hamida N, Liua T, Lua J and Whitea WL (2013): Fucoidan from New Zealand Undaria pinnatifida: Monthly variations and determination of antioxidant activities. Carbohydrate Polymers, 95: 606– 614

26. Marra F, Efsen E, Romanelli RG, Caligiuri a, Pastacaldi S, Batignani G, Bonacchi a, Caporale R, Laffi G and Pinzani M (2002): Ligands of peroxisome proliferator-activated receptor gamma modulate profibrogenic and proinflammatory actions in hepatic stellate cells. Gastroenterology, 119: 466-478.

27. Maruyama H[1], Tamauchi H, Hashimoto M and Nakano T(2003): Antitumor activity and immune response of Mekabu fucoidan extracted from Sporophyll of Undaria pinnatifida. In Vivo, 17 (3): 245-249.

28. Matsubara K, Matsuura Y, Bacic A, Liao M, Hori K and Miyazawa K (2001): Anticoagulant properties of a sulfated galactan preparation from a marine green alga, Codium cylindricum. Int J Biol Macromol, 28: 395-399.

29. McGavin MD and Zachary JF (2003): Pathological Basis of Veterinary 4th edition. Diseases. Mosby Elsevier

30. McLellan DS and Jurd, KM (1992)**:** Anticoagulants from marine algae. Blood Coagulation and Fibrinolysis, 3 (1): 69-77.

31. Notas G, Kisseleva T, rennerand DB and cellsin NKT (2009): liver injury and fibrosis. Clin Immunol, 130: 16–26.

32. Poli G: Pathogenesis of liver fibrosis (2000): role of oxidative stress. Mol Aspects Med, 21: 49-98.

33. Puszczewicz M and Iwaszkiewicz C (2011)**:** Role of anti-citrullinated protein antibodies in diagnosis and prognosis of rheumatoid arthritis. Arch Med Sci, 7 (2): 189–194.

34. Raghavendran H, Sathivel A, Yogeeta R and Devaki T (2007): Efficacy of sargassum polycystum (Phaeophyceae) sulphated polysaccharide against paracetamol-induced DNA fragmentation and modulation of membrane-bound phosphatases during toxic hepatitis. Clinical and Experimental Pharmacology and Physiology, 34: 142–147

35. Reitman S and Frankel S (1957): Transaminase in serum. Smer J Clin Path, 28 : 56.

36. Rubbia-Brandt L, Mentha G, Desmouliere A, Alto Costa AM, Giostra LE and Molas G (1997): Hepatic stellate cells reversibly express alpha-smooth muscle actin during acute hepatic-ischemia. Transplant Proc, 29: 2390–2395.

37. Satoh K (1978): Clinica Chimica Acta, 90: 37-38.

38. Semiha Noyan, Ilkin Cavusolgu and Zehra Minbay F (2006): The effect of vitamin A on CCL_4-induced hepatic injuries in rats: a histochemical, immunohistochemical and ultrastructural study. Acta Histochemica, 107: 421-434.

39. Sener G, Demiralp EE, Cetiner M, Ercan F, Sirvanc S, Gedik N andYegen BC (2006[a])**:** L-carnitine ameliorates methotrexate-induced oxidative organ injury and inhibits leukocyte death. Cell Biol Toxico, 22: 47–60.

40. Sener G, Demiralp EE, Cetiner M, Ercan F and Yegen BC (2006[b]): Beta glucan ameliorates methotrexate-induced oxidative organ injury via its antioxidant and immunomodulatory effects. European Journal of Pharmacology, 542: 170–178.

41. Sweeney EA, Lortat-Jacob H, Priestley GV, Nakamoto B and Papayannopoulou T (2002): Sulfated polysaccharides increase plasma levels of SDF-1 in monkeys and mice: involvement in mobilization of stem/progenitor cells. Blood, 99: 44-51.

42. Tsukada S, Parsons CJ and Rippe RA (2006): Mechanisms of liver fibrosis. Clin Chim Acta, 364: 33-60.

43. Uraz, S, Tahan, V, Aygun, C, Eren, F, Unluguzel, G, Yuksel, M, Senturk, O, Avsar, E, Haklar, G, Celikel, C, Hulagu, S, and Tozun, N (2008): Role of ursodeoxycholic acid in prevention of methotrexate-induced liver toxicity. Dig Dis Sci, 53: 1071–7.

44. Vardi N, Parlakpinar H, Cetin A, Erdogan A, Ozturk C (2010): Protective Effect of Carotene on Methotrexate–Induced Oxidative Liver Damage. Toxicologic Pathology, 38: 592-597

45. Visser K, van der Heijde DM (2009): Risk and management of liver toxicity during methotrexate treatment in rheumatoid and psoriatic arthritis: a systematic review of the literature. Clin Exp Rheumatol, 27: 1017-1025.

46. Wake K (1995)**:** Structure of the sinusoidal wall in the liver. In: E. Wisse, D.L. Knook and K. Wake, Editors. Cells of the Hepatic Sinusoid, The Kupffer Cell Foundation, Leiden: 241–246.

47. West SG (1997): Methotrexate hepatoxicity. Rheum. Dis Clin North Am, 23: 883–915

48. Yang C, Chung D, Shin S, Lee HY, Kim JC and Lee YJ (2003): Effects of molecular weight and hydrolysis conditions on anticancer activity of fucoidans from sporophyll of Undaria pinnatifida. International Journal of Biological Macromolecules, 43: 433–437.

49. Zakim D, Boyer TD: Hepatology (2003): a Textbook of Liver Disease. Fibrosis of the liver: representative molecular elements, and their emerging role as anti-fibrotic targets. Philadelphia: WB Saunder, 347-94.