Therapeutic efficacy of seaweed extract (*Ulva Fasciata* Delile) against invasive candidiasis in mice

Fathy, S.A.[1], Mohamed, M.R.[1], Emam, M.A.[1], Mohamed, S.S.[2], Ghareeb, D.A.[3], Elgohary, S.A.[4] and Abd-El Megeed, D.F.[1*]

[1]Biochemistry Department, Faculty of Science, Ain Shams University, Cairo, Egypt
[2]Microbiology Department, Faculty of Science, Ain Shams University, Cairo, Egypt
[3]Bioscreening and Preclinical Trial Lab, Biochemistry Department, Faculty of Science, Alexandria University, Egypt, Pharmaceutical and Fermentation Industries Development Centre, The City of Scientific Research and Technological Applications, Alexandria, Egypt
[4]Pathology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt
*Corresponding author e-mail: dfsalama@gmail.com

Abstract. *Candida* is the most frequent common causes of invasive fungal infections and associated with high morbidity and mortality. Most of available antifungal agents have side effects. This opened up new avenues to investigate the antifungal efficacy of active extracts from marine algae. So the aim of this study was to evaluate the protective and the curative effect of *Ulva fasciata* extract against an invasive candidiasis in mice and to study its underlying mechanism. The active ingredients of *Ulva fasciata* extract were evaluated using HPLC and GC/MS. Fifty mice were included in current work, and the level of inflammatory markers; Interleukin (IL)-4, IL-12, Interferon-gamma (IFN-γ) and Tumor necrosis factor-alpha (TNF-α) were determined using ELISA kits. Hematological, biochemical and oxidative stress parameters were determined using commercial kits. Moreover, the histopathological examinations were carried on liver, kidney and spleen for all groups. The results obtained showed that treatment with *U. fasciata* either before or after *Candida* infection significantly improved the hematological, biochemical alterations and antioxidant status caused by this infection. Furthermore, the *U. fasciata* reduced histopathological changes induced by *Candida* as well as it could increase the expression of IL-12 and IFN-γ while minimized the expression of TNF-α and IL-4 in all infected mice compared to infected untreated mice. These data propose that *U. fasciata* can ameliorate inflammatory reactions related to *Candida albicans* cytotoxicity via its ability to augment cellular antioxidant defenses by its active compounds.

INTRODUCTION

The polymorphic, diploid yeast *Candida albicans* is one of the most important human pathogenic fungi (Vylkova & Lorenz, 2014). It belongs to ascomycetes division with known potential to threaten life during infections. White asporogenous yeast is a characteristic feature of *Candida* (Chin *et al.*, 2016). As part of the normal human microbiota, *C. albicans* can grow, proliferate and coexist within the human host gastro-intestinal, oropharyngeal or urogenital tract for a long time without causing any symptoms of disease (Wellington *et al.*, 2014). The host

immunity, stress, resident microbiota, and other factors alterations can lead to *C. albicans* overgrowth, causing a wide range of infections ranging from superficial mucosal to hematogenously disseminated candidiasis (Nobile & Johnson, 2015).

The difficulties of managing *Candida* infections include several problems such as toxicity, high cost and limited number of effective antifungal agents; this is in addition to *Candida* resistance to commonly used antifungals, and *Candida* infections relapse. So, the discovery of new natural antifungal agents was necessary (Sasidharan *et al.*, 2011). Freshwater and marine organism's

products have recently become attractive as nutraceutical and functional foods and as a source material for the development of drugs (Soliman *et al.*, 2015). They are considered as potential sources of bioactive secondary metabolites which have important biological activities (Al-Saif *et al.*, 2014).

Ulva fasciata (U. fasciata) is a green algae species of seaweed in the *Ulvaceae* family. The genus *Ulva* is edible, parenchymatous, macroscopic, thread-like seaweed, and an important food source in many Southeast Asian countries. Enteromorpha is a synonymous name of *Ulva* (Farasat *et al.*, 2014). Since, chemical compounds extracted from *Ulva* species have many distinctive biological and pharmacological actions, such as anti-oxidant, antibacterial, anti-hyperlipidemic and anti-peroxidative (Mosaddegh *et al.*, 2014).

Many reports provided special attention for marine algae antibacterial and anti-fungal activities against several pathogens (Rangaiah *et al.*, 2010). Kausalya & Narasimha (2015) proved that the existence of active constituents with antimicrobial activity in extracts of marine algae can be exploited to produce innovate-on drugs for the benefit of the humanity.

So, the recent study aimed to determine the active ingredients of *Ulva fasciata* methanolic extract by using HPLC and GC/MS, in addition to determine the antifungal effect of *Ulva fasciata* methanolic extract against *C. albicans infected* mice.

MATERIALS AND METHODS

Collection of algae

Fresh Chlorophyta (*Ulva fasciata* Delile) was collected from Abu-Qir bay (Alexandria, Egypt) during May and June 2014, at a depth of 1–3 m. The alga was brought to the laboratory in plastic bags containing seawater, to avoid vaporization. The seaweed was identified by Prof. Dr. Mohamed Saad Abd El -Kareem, Professor of Phycology, Botany and Microbiology Department, Faculty of Science, Alexandria University. Then the sample was rinsed with fresh seawater and distilled water to remove associated debris and epiphytes. After that, the cleaned sample was left to dry in the shade at 30°C. The dried sample was finely powdered, using an electric mixing grinder and kept at –20°C until use.

Microorganism

Candida albicans (*C. albicans*) NCPF 8154 (culture collection from Faculty of Agriculture-Ain Shams University) was used as the test organism. The yeast was cultured on Sabouraud dextrose agar at 30°C for 24 h. The stock culture was maintained on Sabouraud dextrose agar slants at 4°C.

Preparation of *U. fasciata* extract

For extraction of bioactive in shade dried seaweed, 100 g of finely powdered alga was added to 400 ml methanol and soaked for 4 days. Removal of the alga from solvent was done by filtration using cheesecloth. After that, the organic extract was concentrated to solvent free by evaporation in a rotary vacuum evaporator (Buchi, B-480) at 40°C and lyophilized to obtain the extract in powder form and dissolved in the respective solvent (Sasidharan *et al.*, 2011).

U. fasciata bioscreening

High performance liquid chromatography analysis (HPLC)

The analysis was carried out for the tested extract at Food Safety and Quality Control Laboratory, Faculty of Agriculture, Cairo University, Egypt using an Agilent Technologies 1260 infinity HPLC Series (Agilant, USA). A 2.0 g amount of alga extract was accurately weighed and soaked into 5 ml of methanol/water 80/20 (V/V) extraction solution for 24 hr. Afterwards, the sample was centrifuged at 200 rpm. An aliquot of the supernatant phase was evaporated then dissolved in HPLC grade solvent to be ready for injection into the HPLC system. The Agilent Technologies 1260 infinity HPLC Series was equipped with an Autosampler and Diode Array Detector VWD detector. The analytical column was Agilent ZORBAX Eclipse plus C18, 100 mm ×4.6 mm i.d, 5µm. The separation is reached by a ternary linear

elution gradient with (A) 0.2 % H_3PO_4/ water (v/v), (B) methanol and (C) acetonitrile with flow rate 1 ml/min.

Gas chromatography-mass spectrometry qualitative analysis (GC/MS)

The GC-MS system (Agilent Technologies) was supplied with a gas chromatograph (7890B) and mass spectrometer detector (5977A) which carried out for the tested extract at the Central Laboratories Network, National Research Centre, Cairo, Egypt. 1.0 g of the tested extract was dissolved in hexane then filtered using syringe filter, after that transferred to GC vial for GC/MS analysis. The gas chromatography was equipped with HP-5MS column (30 m x 0.25 mm interior diameter and 0.25 µm film thickness). Analyses were done with helium as the transporter gas at a flow rate of 1.5 ml/min at a split ratio of 1:10, injection quantity of 1 µl and the subsequent temperature program: 60°C for 1 min; rising at 5°C /min to 280°C and held for 10 min. The injector and detector were remained at 280°C and 290°C. Mass spectra were achieved by electron ionization voltage (EI) at 70 eV and using a spectral range of m/z 40-550. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

Anticandidal activity of *U. fasciata* extract

Experimental animals

Following the prior *in vitro* testing of the antifungal activity of *U. fasciata* extract (Fathy *et al.*, 2017) an *in vivo* verification was conducted. Fifty male albino mice (25-35 g, 80 days old) were used throughout the study. Mice were obtained from the animal house unit, Medical Research Centre (MRC), Faculty of Medicine, Ain Shams University. The animals were housed in a temperature-controlled room at 25°C with alternating 12 h-light/12 h-dark cycles. All animals were acclimatized for 7 days before the beginning of the experiment and were fed with standard animal feed and water. The experimental protocols were approved by the Institutional Ethical Committee of Faculty of Medicine, Ain Shams University, Egypt. The study was conducted in accordance with the internationally accepted principles for laboratory animal use and cares as found in for example the European Community guidelines (EEC Directive of 1986; 86/609/EEC) or the US guidelines (NIH publication no. 85-23, revised in 1985).

Experimental design

All treatments started at the same time and lasted for the specified duration. Mice were equally divided into five groups as follows: The normal control group (G1) was injected intra-peritoneally (i.p.) with a vehicle solution (0.25 ml, 20% polyethylene glycol (PEG) daily for seven days). The *C. albicans* group (G2) received a single dose intravenous injection of *C. albicans* into the lateral tail vein of mice (0.1 ml of 1×10^7 CFU/ml PBS). *U. fasciata* group (G3) received i.p. injections of the *U. fasciata* extract only (0.25 ml, 200 mg in 20% PEG/ Kg body weight/day) for 7 days (Abirami & Kowsalya, 2012). *U. fasciata+C. albicans* protective group (G4) received *U. fasciata* extract for 7 days i.p then injected by *C. albicans* after one hour of the last dose of the extract as mentioned above. *C. albicans+ U. fasciata* treated group (G5) received *U. fasciata* extract after 24 h single dose of *C. albicans* for 7 days.

At the end of the experiment, blood was collected and mice were sacrificed by carotid bleeding under ether anesthesia. Liver divided into parts, where one part was used for nitric oxide and malondialdehyde determination, while the rest of liver, kidney and spleen were fixed in 10% formalin for histopathological investigation.

Hematological and biochemical parameter measurements

Blood was collected in EDTA and heparin tubes for hematological and biochemical parameters, respectively for all examined mice. The collected blood on heparin tubes was centrifuged at 3000 rpm for 10 min. then the obtained plasma was kept at -80°C until used.

For hematological measurements, CBC was conducted on a Blood Cell Analyzer [Orphe CBC automatic analyzer (France)]

according to the manufacturer's instructions. The number of leucocyte (WBC), erythrocyte (RBC), lymphocytes (LYM), neutrophilic granulocytes (GRA), and platelets (PLT) were counted.

Plasma alanine transaminase (ALT) and aspartate transaminase (AST) activities were determined according to the procedure described by Reitman & Frankel (1957). ALT catalyzes the formation of pyruvate from L-alanine and α-oxoglutarate. The pyruvate which formed was assayed by the formation of 2, 4-dinitrophenylhydrazine pyruvate derivative which yields a reddish-brown color in alkaline medium. In addition, AST catalyzes the formation of oxaloacetate from the reaction of L-aspartate and α-oxoglutarate. The formed oxaloacetate was assayed by the formation of 2, 4-dinitrophenyl hydrazinone oxaloacetate derivative which yields a reddish-brown color in alkaline medium.

Plasma albumin was measured according to the procedure of Doumans et al. (1971), where albumin was added to a buffered solution of bromocresol green dye, a blue color appears and is proportional to the albumin concentration in the sample. Total protein was determined in plasma according to method described by Gornal et al. (1949), in which the protein produced a violet color in the presence of an alkaline cupric sulfate; the intensity of the colour is proportional to their concentration.

Plasma urea concentration was determined according to the method described by Searcy et al. (1967), where urea was hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide. The ammonia ions react with hypochloride and salicylate to give a green dye. Plasma creatinine concentration was determined according to the method described by Fabiny & Ertingshausen (1971), where creatinine reacted with alkaline picrate to form creatinine picrate with an amber color.

Assessment of oxidative stress markers
Lipid peroxidation was assessed as the amount of thiobarbituric acid reactive substances (TBARS) determined by the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA) as defined by Ohkawa et al. (1979).

Total antioxidant capacity (TAC) was measured according to the methodology of Koracevic et al. (2001), where the determination of the antioxidative capacity was performed by the reaction of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide (H_2O_2). The antioxidants in the sample eliminated a certain amount of the provided hydrogen peroxide. The residual H_2O_2 was determined colorimetrically by an enzymatic reaction which involved the conversion of 3, 5 dichloro-2-hydroxy benzene-sulphonate to a colored product.

Nitric oxide (NO) was determined in accordance with the procedure of Montgomery & Dymock (1961), This assay depended on the conversion of nitrate to nitrite via nitrate reductase. Then, nitrite in acidic medium produced a nitrosating agent which reacted with sulfanilic acid to produce the diazonium ion. This product was then coupled to N-(1-naphthyl) ethylenediamine to form a bright reddish – purple color azo-derivative which absorbed light at 540 nm.

Assessment of inflammatory markers
Interferon-gamma (IFN-γ), Interleukins 4, 12 (IL-4, IL-12), and Tumor necrosis factor-alpha (TNF-α) were determined using ELISA kits according to the instructions of the manufacturers (Bioneovan Company, China). Concentrations of cytokines were determined using a standard curve and expressed in pg/ml.

Histopathological examinations
Tissue specimens were cleared in xylene and embedded in paraffin at 56°C in hot air oven for 24 h. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin (H&E) stains and Periodic acid-Schiff (PAS) stain for histopathological examination using the electric light microscope (Sasidharan et al., 2007).

Statistical analysis

All data were statistically evaluated by one-way analysis of variance (ANOVA) and Least Signicant Difference (LSD) using SPSS software version 16. The experimental results were expressed as the mean ± standard error (SE). The difference was considered statistically significant at $p < 0.05$.

RESULTS

The HPLC analysis of the crude powder of *U. fasciata* methanolic extract indicated the presence of several kinds of phenolic compounds, where benzoic acid and gallic acid were the main ones, beside that several other phenolic compounds were detected like; salicylic, cinnamic, vanillic, ellagic acid and rutin as shown in Table 1. In addition, the GC/MS analysis of *Ulva fasciata* extract revealed the presence of 54.5% fatty acids [18.2% saturated fatty acids (SAFA), 22.7% monounsaturated fatty acid (MUFA), 4.5% polyunsaturated fatty acids (PUFA, ω6) and 9.1% polyunsaturated fatty acids (PUFA, ω3)], while the remaining part was nonpolar compounds (Table 2).

Infection with *C. albicans* significantly decreased ($p < 0.05$) red blood cells (RBCs) count, hemoglobin and white blood cells (WBCs) count with non-significant reduction in platelet count when compared to the normal control group as shown in Table 3. WBC differential count showed an increase of lymphocytes and the concomitant decrease of monocytes and granulocytes. On the other hand, the treatments with *Ulva* extract (even after or before) successfully improved the *C. albicans* induced alterations in these hematological parameters. In addition, the administration of *U. fasciata* to healthy animals showed non-significant effect on the normal complete blood picture and differential leukocyte count as compared to a normal control group (Table 3), which indicated that the *U. fasciata* had no adverse effects on circulating blood cells.

There were significant elevations in plasma ALT ($p < 0.05$), AST ($p < 0.01$), urea ($p < 0.01$) and creatinine ($p < 0.05$) levels accompanied by significant depletion ($p < 0.01$) in total protein and ($p < 0.05$) albumin levels in the *Candida* infected group compared with the normal control group (Table 4). Administration of *U. fasciata* extract to mice for 7 days, even after or before infection successfully reversed the hepatic and renal damage exerted by *C. albicans*.

As shown in Table 4, there was a significant elevation ($p < 0.001$) in NO and MDA levels accompanied with a significant

Table 1. *Ulva fasciata* methanolic extract phenolic compounds

Phenolic compound	RT(min)	Area [mAU×s]	Conc. (mg/kg)
Gallic acid	4.578	133.18544	50.88
Catechol	8.783	2.07572	2.46
p-Hydroxy benzoic acid	10.240	27.48520	28.44
Vanillic acid	11.553	6.03090	3.85
Caffeic acid	11.812	–	ND
Syringic acid	12.032	5.25065	2.73
Vanillin	13.600	–	ND
p-Coumaric acid	14.884	17.19336	1.82
Ferulic acid	15.733	9.53595	3.30
Rutin	16.745	5.81182	3.29
Ellagic acid	17.254	13.84077	11.30
Benzoic acid	17.708	15.14842	75.45
o-Coumaric acid	18.719	21.12771	8.12
Salicylic acid	20.597	7.80115	4.40
Cinnamic acid	23.125	45.19955	3.70

RT: Retention time
ND: Not detected

Table 2. GC/MS of *Ulva fasciata* extract

Peak	RT^a (min)	Compounds	Area	C$_F$^{b}
1	22.179	E-14-Hexadecenal	635822.99	$C_{16}H_{30}O$
2	25.565	7,11,15-Trimethyl-3-methylidenehexadec-1-ene	3560107.85	$C_{20}H_{38}$
3	25.699	2-Pentadecanone,6,10,14-trimethyl	743936.19	$C_{18}H_{36}O$
4	26.072	3,7,11,15-Tetramethyl-2-hexadecenyl Acetate	814931.3	$C_{22}H_{42}O_2$
5	27.314	Hexadecanoic acid,methyl ester	2271156.58	$C_{17}H_{34}O_2$
6	27.477	Cis-5,8,11,14,17-Eicosapentaenoic acid	655146.98	$C_{20}H_{30}O_2$
7	27.663	(9Z)-Hexadec-9-enoic acid	1565657.32	$C_{16}H_{30}O_2$
8	28.206	n-Hexadecanoic acid	63439962.76	$C_{16}H_{32}O_2$
9	30.584	9-Ocatadecanoic acid,methyl ester,(E)	1171979.05	$C_{19}H_{36}O_2$
10	30.811	(2E,7R,11R)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol	3201983.05	$C_{20}H_{40}O$
11	31.073	Cis-5,8,11,14,17-Eicosapentaenoic acid	1448591.3	$C_{20}H_{30}O_2$
12	31.196	9,12- Octadecadienoic acid	2190985.31	$C_{18}H_{32}O_2$
13	31.312	9,12,15-Octadecatrienoic acid,(Z,Z,Z)	4439569.9	$C_{18}H_{30}O_2$
14	31.394	(Z)-octadec-11-enoic acid	9891336.86	$C_{18}H_{34}O_2$
15	32.023	9-Octadecanoic acid	663630.27	$C_{18}H_{34}O_2$
16	33.224	Hexadecanoyl chloride	2019819.53	$C_{16}H_{31}ClO$
17	35.043	9-Octadecenamide,(Z)	3124775.98	$C_{18}H_{35}NO$
18	36.966	Hexadecanoic acid,1-(hydroxyl methyl)-1, 2 ethanediyl ester	1893812.4	$C_{35}H_{68}O_5$
19	41.314	Hexasiloxane,1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	1026773.22	$C_{12}H_{38}O_5Si_6$
20	49.37	Stigmasta-5,24(28)-dien-3-ol,(3 beta,24Z)	14404641.37	$C_{29}H_{48}O$
21	52.29	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15, 15-hexadecamethyl-	1226678.96	$C_{16}H_{50}O_7Si_8$
22	53.525	Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13, 13-tetradecamethyl	2700704.96	$C_{14}H_{44}O_6Si_7$

a: retention time.
b: compound formula.

Table 3. Effect of *U. fasciata* extract on hematological parameters in *C. albicans* infected mice

	Normal control	*C. albicans*	*U. fasciata*	*U. fasciata* + *C. albicans*	*C. albicans* + *U. fasciata*
WBCs ($\times 10^3$/µl)	15.43±0.27	7.46±1.39^a	15.35±1.07^b	10.6±1.27^{a,b}	14.0±0.98^b
Haemoglobin (gm/dl)	14.01±0.15	12.27±0.13^a	16.53±1.12^b	15.40±0.50^b	12.90±0.75^a
Platelets ($\times 10^3$/µl)	355.50±29.48	308.0±17.78	406.0±23.44^b	403.0±23.27^b	535.93±23.88^{a,b}
RBCs ($\times 10^6$/L)	8.9±0.65	5.86±0.25^a	8.57±0.38^b	9.22±0.35^b	8.42±0.36^b
Granulocytes %	7.0±1.5	4.2±0.3^a	7.3±0.4^b	7.6±2.4^b	8.3±1.8^b
Lymphocytes %	68.0±2.3	74.8±1.6^a	62.0±1.9^b	67.9±1.4^b	63.2±1.2^b
Monocytes %	27.4±4.7	20.3±0.9^a	31.6±0.9^b	24.5±1.0^b	28.5±1.5^b

Values are presented as mean ± SE for 10 animals in each group.
WBCs: White blood cells, RBCs: Red blood cells.
a: Significant difference at $p< 0.05$ compared to the normal control group.
b: Significant difference at $p< 0.05$ compared to the infected group.

Table 4. Effect of *U. fasciata* extract on biochemical parameters in *C. albicans* infected mice

	Normal control	*C. albicans*	*U. fasciata*	*U. fasciata* + *C. albicans*	*C. albicans* + *U. fasciata*
ALT (U/L)	41.0±12.5	117.0±13.3^a	31.3±3.3^b	53.8±3.5^b	79.5±6.1^b
AST (U/L)	170.7±13.6	313.0±32.9^a	154.3±14.3^b	210±2.2^b	238.3±34.6^b
Total protein (g/dl)	6.5±0.3	4.7±0.4^a	5.2±0.1^b	6.1±0.5^b	6.2±0.3^b
Albumin (g/dl)	0.27±0.02	2.4±0.07^a	2.9±0.07^b	2.6±0.08^b	2.7±0.07^b
Urea (mg/dl)	18.3±0.8	33.3±1.9^a	16.3±0.88^b	19.6±1.2^b	25.3±2.4^b
Creatinine (mg/dl)	0.27±0.02	0.4±0.04^a	0.16±0.01^b	0.22±0.02^b	0.28±0.01^b
MDA (nmol/g tissue)	18.3±0.8	33.3±1.9^a	16.3±0.8^b	19.6±1.2^b	25.3±2.4^b
TAC (mM/L)	1.4±0.04	0.6±0.04^a	1.97±0.1^b	1.9±0.05^b	1.87±0.1^b
NO (µmol/L)	1.03±0.2	22.9±1.3^a	3.06±0.4^b	7.3±0.4^b	3.7±0.2^b

Values are presented as mean ± SE for 10 animals in each group.
ALT: alanine transaminase, AST: aspartate transaminase, MDA: Malondialdehyde, TCA: Total antioxidant capacity and NO: Nitric oxide.
a: Significant difference at p< 0.05 compared to the normal control group.
b: Significant difference at p< 0.05 compared to the infected group.

depletion (p<0.001) of TAC in *Candida* infected group compared with the control group, whereas both *U. fasciata* protected and treated groups significantly increased the level of TAC, while a significant decreased in MDA and NO levels was observed as compared to an infected group.

In addition, there was a significant increase (p<0.001) in both IL-4 and TNF-α levels that associated with a significant decrease in both (p<0.05) IL-12 and (p<0.01) IFN-γ levels in the infected mice when compared to the normal control group (Figure 1). While, the *U. fasciata* extract administrations (G4 & G5) could successfully normalize both pro and anti-inflammatory measured cytokines compared to the infected group.

In order to assess the cytoprotective effect of *U. fasciata* against *C. albicans*-induced organs pathological injuries, histopathological examination of liver, kidney and spleen tissue specimens was done. In the normal control group, no histopathological alterations were observed in tested organs (Figure 2, panel a, b, c). However, the *C. albicans* infected mice showed various pathologic alterations; the kidney showed glomerulosclerosis with obsolescent glomeruli, atrophic tubules with hyaline casts, interstitial chronic inflammation and fibrosis with occasional scattered *Candida* hyphae (Figure 2, panel d). Moreover, the liver showed slightly disturbed architecture with scattered foci of spotty necrosis and interface activity, also there were expanded fibrotic portal tracts showing moderate lymphocytic infiltration with porto-portal and porto-central bridges (Figure 2, panel e). In addition, spleen showed lymphoid hyperplasia with scattered multinucleated giant cells and a moderate congestion with expanded red pulp (Figure 2, panel f). Mice receiving *U. fasciata* alone showed no histopathological alterations in these tested organs. Also, there were mild histopathological changes in these organs in group (4) (protected group with extract) as a result of infection with *Candida*. However, mice receiving treatment of *U. fasciata* after the infection showed an improvement in these changes near to normal structure (Figure 2, panels g, h, i).

DISCUSSION

Seaweeds have many beneficial nutritive bioactive compounds such as pigments, vitamins, polyphenols, fibers, minerals and polysaccharides (Ismail, 2017). Also, they are low in fat and calories with high levels of essential fatty acids and amino acids besides about 80-90% water. *Ulva* was reported as a rich source of antioxidant compounds as they include several types of phenolic
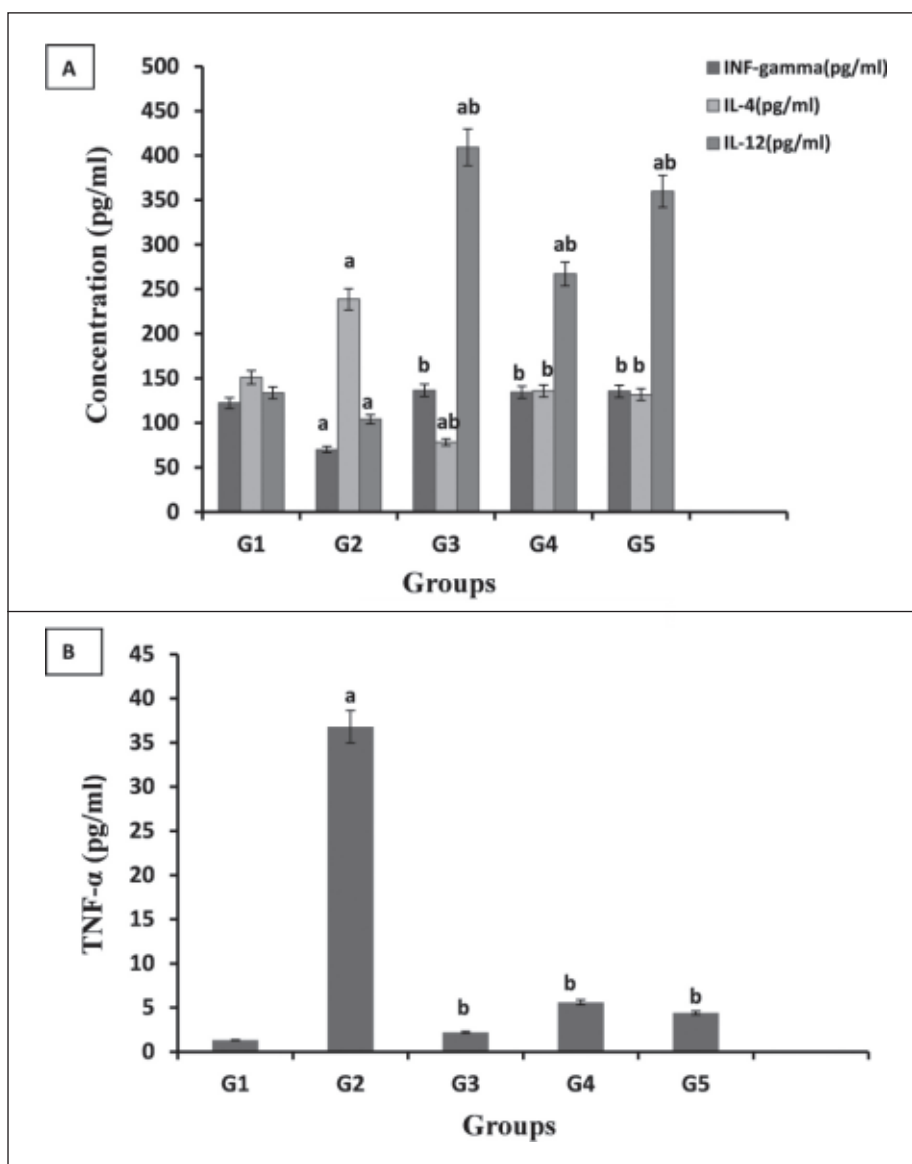
Figure 1. Effect of *Ulva fasciata* extract administration on inflammatory markers concentrations in *C. albicans* infected mice. A; IL-4, IL-12 and IFN-γ, B; TNF-α. Values are presented as mean ± SE for 10 animals in each group. G1, normal control group; G2, *Candida*-infected group; G3, algae control group; G4, mice pre-treated with extract before infection; G5, mice treated with extract after infection. IFN-γ: Interferon gamma, IL-4: Interleukin-4, IL-12: Interleukin 12 and TNF-α: Tumor necrosis factor alpha. a: Significant difference at $p < 0.05$ compared to the normal control group (G1). b: Significant difference at $p < 0.05$ compared to the infected group (G2).

compounds (Farag *et al.*, 2003). The HPLC results obtained from this study showed that benzoic acid and gallic acid were the major ones between the phenolic compounds of *U. fasciata* methanolic extract. The presence of great content of these phenolic compounds confirmed the ability of extract to have antimicrobial activity due to the existence of a methoxy (OCH_3) group in the meta position of the benzene ring, carboxylic acid (COOH) and also two hydroxyl (OH) groups in para and ortho positions appeared to be essential
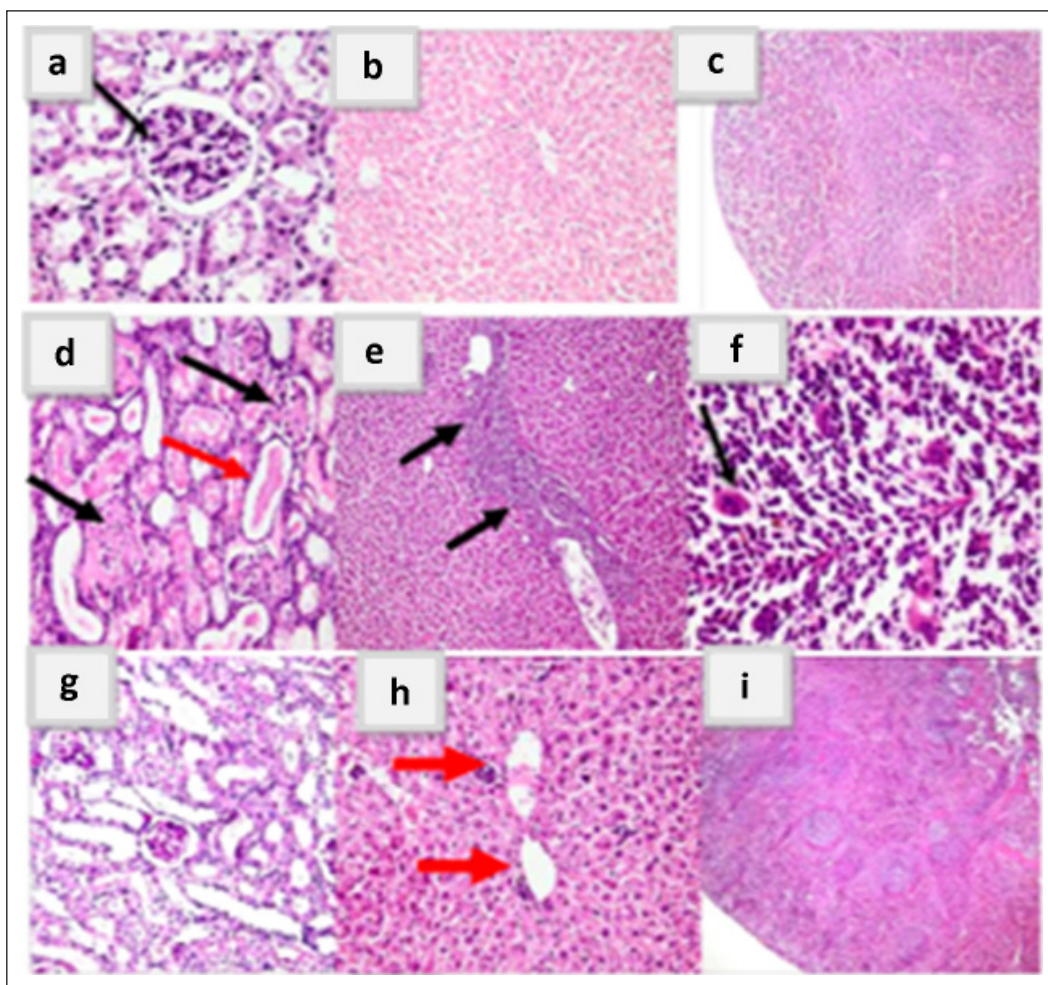
Figure 2. Effects of *Ulva fasciata* extract administration on *Candida*-induced histopathological changes in mouse liver, kidney and spleen. a) Kidney section of normal control group showing normal renal parenchyma with well-formed glomeruli "black arrow" (H&E and PAS stain x400), b) Section of the liver in (normal control group) showing preserved architecture (H&E stain x40). c). Section of the spleen in (normal control group) showing well-formed white and red pulps (H&E stain x40). d) Section of kidney in group (2) (infected untreated control group) showing sclerotic glomeruli "black arrows" with hyaline casts "red arrow" (H&E stain x200). e) Section of liver in group (2) showing expanded portal tracts with marked inflammation and porto-central bridging with interface activity "black arrow" (H&E stain x100), f) Section of spleen in group (2) showed scattered multinucleated giant cells "black arrow" (H&E stain x400). g) Section of kidney in group (5) (infected treated with extract) showing scattered normal glomeruli (PAS stain x200). h) Section of liver in group (5) showed mild inflammatory infiltrate "red arrows" (H&E stain x200). i) Section of spleen in group (5) showed congested with mild lymphoid hyperplasia (H&E stain x40).

for anti-microbial activity (Alves *et al.*, 2013). In addition, the GC/MS examination of *U. fasciata* (Table 2) showed the presence of 54% of fatty acid among them the mono-unsaturated and saturated fatty acids represent the highest percentage. The high content of these fatty acids in *U. fasciata*

methanolic extract also confirmed its antimicrobial activity due to a close relation between the structure of fatty acids and their capability of function as antimicrobial agents (McGaw *et al.*, 2002).

Previous study reported that these compounds have antifungal activity alone

or combined with each other (Teodoro *et al.*, 2015). In addition, Shobier *et al.* (2016) revealed that the *U. fasciata* extract has been found to comprise 28 compounds which classified as follows; 82.108% saturated fatty acids (SAFA), 14.72% monounsaturated fatty acid (MUFA), 1.987% polyunsaturated fatty acids (PUFA) ω6 and 1.19% polyunsaturated fatty acids (PUFA) ω3.

So, the recent study was designed to examine the potential prophylactic effects of the *U. fasciata* methanolic extract against oxidative stress resulted by *C. albicans* in mice. The experimental models assessing host defence against *C. albicans* have revealed that innate and acquired cell-mediated immunity was deeply involved in the anti-*Candida* reaction (Van't *et al.*, 1992).

In the present work, the intravenous injection of *C. albicans* significantly altered the hematological parameters, while the treatment with *Ulva* extract was able to normalize all the hematological parameters and also was capable of inverting the differential count of leukocyte by elevating the percentage of granulocytes which could internalize fungus on the initial days of infection, this action may be due to the presence of active compounds in the *Ulva* extract. These results highlighted the potency of *Ulva* extract as a curative agent in candidiasis.

In parallel with the current study, Margret *et al.* (2009) found that *U. lactuca* extract successfully maintained the hematological parameters in a normal range compared with a control group; also Abirami *et al.* (2012) stated that the treatment with *U. fasciata* restored all the altered hematological parameters to almost near normal in a tumor induced mice. This may be due to the iron content of the seaweeds. Also, the current HPLC and GC/MS analysis of *U. fasciata* indicated the existence of cinnamic and oleic acid, where cinnamic acid was previously reported to have an immune regulatory effect against *C. albicans* by monocytes activation, and oleic acid could enhance neutrophil phagocytic capacity (Conti *et al.*, 2013).

The current study showed a significant elevation in urea, creatinine, ALT, AST as well as MDA with a concomitant decrease in total protein, albumin and TAC levels in *Candida* infected group compared to a normal control group. While, *U. fasciata* intake before or after infection could improve the renal, hepatic damage and MDA levels caused by *C. albicans*, which may be due to the ability of *U. fasciata* antioxidant components to protect the biomembranes from lipid peroxidation and to boost cellular anti-oxidant defenses.

In healthy kidneys, urea is further concentrated and sequestered in collecting ducts, but in case of fungal infection, tissue necrosis, and the host inflammatory response may cause accumulation of urea, which further enhances virulence (Dhammika *et al.*, 2012). Furthermore, Lipid peroxidation of hepatocytes is the primary mechanism associated with liver damage after *Candida* inoculation of mice. Normal injury raises portal and systemic endotoxin levels as well as translocation to the liver, which subsequently causes neutrophils recruitment and the further release of reactive oxygen species (ROS). Lipid peroxidation of membrane phospholipids raised permeability of hepatocyte that leaded to the outflow of cellular enzymes in blood (Cheng *et al.*, 2014).

The results obtained by Cheng *et al.* (2014) indicated that 8 phenolic acids (ellagic, ferulic, gallic, *p*-coumaric, *p*-hydroxybenzoic acids, caffeic, proto-catechuic, and chlorogenic) in jujube honey proved strong free radical-scavenging possibility, lipid peroxidation inhibition, and increasing antioxidant activity, so it was effective in an inhibition of chronic hepatotoxicity induced by alcohol in mice and may be used as a hepatoprotective agent. In same line, it was reported that administration of *U. fasciata* methanolic extract to cancer bearing mice could significantly return the elevated levels of liver enzymes and tissue damage to the normal (Abirami *et al.*, 2012). Beside, Akdemir *et al.* (2017) who reported the protecting effect of *p*-coumaric acid on acute kidney and liver damages caused by Cisplatin and decreasing liver MDA, which may be due to the repressive effect of *p*-coumaric acid on lipid peroxidation by

advantage of its anti-lipid peroxidation feature.

The present results proved that *C. albicans* inoculation enhanced a severe inflammation as characterized by significant elevations in NO and protein expression levels of IL-4, and TNF-α, while the depletion in IL-12 and IFN-γ. While the treatment with the *U. fasciata* extract showed anti-inflammatory and antioxidant activities, where it could normalize all the inflammatory measured parameters. This action may be attributable to its content of phenolic compounds and other active compounds which possess important anti-inflammatory activities as indicated by HPLC and GC/MS analysis data of the present study.

It was reported that during *Candida* infection, *C. albicans* adhesion to host epithelial cells was believed to include sequences of interactions, lead to epithelial cells production of a variety of cytokines, chemokines and a diversity of antimicrobial peptides. In addition, the rapid response of epithelial cells to the incidence of any form of the fungus, stimulating different intracellular signaling pathways, including Nuclear Factor-kappa (NF-κB) pathway (David *et al.*, 2015). The nuclear factor NF-κB characterized a group of structurally related proteins, which combined to create hetero- or homo-dimers as DNA-binding forms**,** it played evolutionarily preserved and important roles in the activating and arrangement of both adaptive and innate immune responses by regulating the ex-pression of a variety of genes vital for immune functions, including pro-inflammatory cytokines, for example IL-1 and TNF-α, chemokines, and adhesion molecules (Qiang *et al.*, 2003). TNF-α considered as a key cytokine in inflammatory reactions because it was formed in the initial step of inflam-mation, which in turn switched the production of further cytokines (Locksley *et al.*, 2001).

Moreover, earlier *in vitro* and *in vivo* studies have revealed that polymorpho-nuclear leukocytes and/or macrophage antifungal activities were controlled by cytokines (Brieland *et al.*, 2001). Specifi-cally, pro-inflammatory cytokines (TNF-α and/or IFN-γ) which have been shown to play key roles in host defense aganist *C. albicans* infections, due to their ability to enhance phagocytosis of *C. albicans* blastoconidia and increase oxygen-dependent and independent candicidal activity. In contrast, the production of anti-inflammatory cytokines such as IL-4 and IL-10 which weaken development of a protective immune response to *C. albicans*, due to the down regulation of phagocytic cell effector mechanisms. IL-12 is an important immunoregulatory cytokine that is produced mainly by antigen-presenting cells. IL-12 induces IFN-γ production and triggers CD4+ T cells to differentiate into T helper cells type 1 (Th1) cells (Brieland *et al.*, 2001).

In the same line, previous studies reported that infection with *C. albicans* associated with high levels of pro-inflammatory monocyte derived cytokines such as TNF-α and IL-4, which lead to suppression of immunity against candidiasis (Stark, 1980). Besides, Hajyani & Modaresi (2016) investigated that the infection is related to Th2, which cause production of IL-10 and IL-4 and interfere in performance of Th1 cells and IFN-γ.

Also, Khan *et al.* (2003) stated that the candidacidal pathway in murine neutrophils is nitric oxide (NO) dependent. Where the current investigation showed that the inoculation with *C. albicans* significantly increased oxidative stress by increasing NO level that results from the excessive production of ROS that exhaust antioxidant defenses. As in candidiasis, the production of NO is greatly increased due to the induction of inducible nitric oxide synthase (iNOS) by pro-inflammatory cytokines leading to elevated levels of NO (Soliman *et al.*, 2015).

Abd El-Aty *et al.* (2014) pointed out to the presence of a positive relation between antioxidant activity and amount of phenolic compounds in the crude extracts. Moreover, Al-Saif *et al.* (2014) revealed that carotenoids, flavonoids and polyphenols of marine algae could protect the body's tissues from oxidative stress and related pathologies like inflammation and cancer, besides Shobier *et al.* (2016) found that most of the com-ponents were identified from *U. lactuca* and *U. fasciata* possess antimicrobial

and antioxidant activities. According to Chakraborty *et al*. (2013), seaweeds phenolic compounds can chelate metal ions and prevent radical formation, therefore improving the antioxidant intrinsic coordination.

Previous findings stated that the alcoholic extract of *U. fasciata* collected from Gujarat Coast exhibited anti-inflammatory and antiviral activities (Pandey *et al*., 1988), besides Margret *et al*. (2009) reported that *U. lactuca* methanolic extract collected from Tuticorin Coast showed high anti-inflammatory activity, due to its ability to prevent the production of inflammation mediators such as histamine, prostaglandin and serotonin. Also, Abd-Ellatef *et al*. (2017) suggested that the anti-inflammatory activity of the marine alga *Gracillaria verrucosa* was due to its inhibitory effects on the production of some pro-inflammatory mediators (TNF-α, NO, and IL-6).

As shown in the current GC/MS results, the tested extract contained several compounds that previously reported to possess an anti-inflammatory activity like phytol, oleic acid, and n-hexadecanoic acid (Aparna *et al*., 2012). In addition, the anti-inflammatory function of the phenolic antioxidants could be explained by the inhibition of NF-κB through prevention of DNA-binding to NF-κB and thus stopping the cascade of inflammatory reactions by inhibition of TNF-α and other pro-inflammatory cytokines expression (Qiang *et al*., 2003).

The current results were confirmed by the histopathological findings, using sections of liver, spleen, and kidney, where *C. albicans* causes obvious histopathological alterations and degenerative changes of the tested organs structures, whereas the *Ulva* extract supplementation could successfully protect the organs from damage induced by invasive candidiasis which may due to its ability to prevent cohesion of *C. albicans* to host epithelial cells and formation of hypha.

Previous studies reported extensive histopathological lesions in mice infected with *C. albicans*, with multiple foci of hyphal invasion in kidney, heart, brain, and spleen. In kidney, there were largest and most numerous foci, with a cortical perivascular or periglomerular distribution, and were accompanied with minimal to severe mononuclear cell inflammatory infiltrates after 24 h of infection. Hyphae were also visible in spleen of infected mice after 48 h of infection and were accompanied with mild inflammatory cell infiltrates (Brieland *et al*., 2001). Hamzia (2016) stated that *C. albicans* infected mice, had necrosis and fibrosis in spleen cells, hemorrhage, and sinusoided sinuses in hepatocytes, and finally, hemorrhage and necrosis in kidney cells. In addition, it was found that mice infected with *C. albicans* and supplemented with extract of brown algae *Ascophyllum nodosum*, the spleen has showed huge number of megakaryocyte; and liver has shown a mild hemorrhage and hydropic degeneration; while the tissue of kidney appeared to be a normal tissue and showed almost no changes.

CONCLUSION

The results obtained from this study demonstrated the protective effect of *Ulva fasciata* methanolic extract against *Candida albicans*-induced oxidative stress and the resultant organ dysfunction in mice. This prophylactic effect maybe induced *via* its content of wide range of active compounds which possess important anti-inflammatory, anti-microbial, antioxidant activities, and they have the ability to increase the expression of the antioxidant defense systems and to inhibit the expression of TNF-α and other pro-inflammatory cytokines, thus stopping the cascade of inflammatory reactions. The demonstrated protective anticandidal effects of *Ulva fasciata* make it a good candidate for future use in preventing and treating candidiasis. Further research is required to isolate the active components of *Ulva fasciata* and examinee their anticandidal activities.

Conflict of interest
The authors declare that no conflict of interest.

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