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Cystoseira myrica and *Padina pavonica*: A potential natural hope against hepatic injury in animal model

Hanaa H.Ahmed[1]*,Muhammed M.Hegazi[2] and Christeen B.Fahim[2]

[1]*Hormones Department, National Research Centre, Dokki, Giza, Egypt, Affiliation ID:60014618*
[2]*Marine-Science Department, Faculty of Science, Suez Canal University,41522 Ismailia, Egypt*

ABSTRACT

The current study was undertaken to investigate the potency of two species of brown algae extracts in mitigating thioacetamide-induced liver injury in rats. This study was conducted on six groups, the first group served as negative control and the groups starting from (2-6) were administered with thioacetamide; the second group was left untreated and served as positive control group, group 3 and 4 were orally treated with Cystoseria myrica fresh and dry extracts respectively, whereas group 5 and 6 were orally administered with Padina pavonica fresh and dry extracts respectively. Thioacetamide intoxicated group administered with fresh or dry extracts of Cystoseria myrica or Padina pavonica displayed significant inhibition in liver enzymatic activity in serum. Also, the treatment with fresh or dry Cystoseria myrica or dry Padina pavonica extract resulted in significant decline in serum cholesterol and LDL levels. Of note, serum HDL level exhibited significant elevation in the groups treated with dry extract of Cystoseria myrica. The rat groups receiving dry extract of Cystoseria myrica or Padina pavonica showed significant reduction in plasma H2O2 level, while those treated with fresh or dry Cystoseria myrica or dry Padina pavonica extract displayed significant depletion in hepatic H2O2 level. Plasma total antioxidant capacity was found to be significantly increased in the rat groups administered with fresh or dry Cystoseria myrica or dry Padina pavonica extract whereas, hepatic level of total antioxidant capacity recorded significant increase in rats treated with fresh or dry extract of Cystoseria myrica or Padina pavonica. Worth mentioning , the rat groups treated with dry or fresh Cystoseria myrica or dry Padina pavonica extract showed significant depletion in serum TNF-α level. In view of the present results, this study introduces a promising insight on the alleviating role of Cystoseria myrica and Padina pavonica extracts against hepatotoxicity induced in rats owing to their hepatoprotective, hypolipidemic, antioxidant and anti-inflammatory actions.

Keywords: *Cystoseira myrica*, *Padina pavonica*, Fucoidan, Hepatotoxicity, Rat.

INTRODUCTION

Marine seaweeds are a valuable food resource which contains low calories, and they are rich in vitamins, minerals, proteins, polysaccharides, phenols, steroids and dietary fiber[1]**.** There are a number of reports regarding the medicinal importance of seaweeds belonging to Phaeophyceae, Rhodophyceae and Chlorophyceae from all over the world [2,3]. They are considered as potential source of bioactive metabolites for pharmaceutical industry and drug development as seaweeds are able to generate a wide range of secondary metabolites (biologically active) that are not found in other organisms. These compounds are produced in response to situations of oxidation and extreme environmental conditions in which they live [4].

Brown algae are a large group of mostly marine multicellular algaewhich are a rich source of various active ingredients including polyphenols and different polysaccharides, namely alginates, laminarins and fucoidans [5,6].

Extracts from brown algae have been reported as anticoagulant [7], antiviral [8], anti- thrombogenic, anti-tumor [9], anti- inflammatory[10]and antioxidant besides immunomodulatory activities [11].

Liver fibrosis is the excessive accumulation of extracellular matrix proteins including collagen that occurs in most types of chronic liver diseases. Advanced liver fibrosis results in cirrhosis, liver failure and portal hypertension which ultimately require liver transplantation. Liver fibrosis and cirrhosis are now among the top ten causes of death worldwide and liver diseasesareamong the top five causes of death in middle-age in many developed countries [12].

So far, no therapeutic intervention has been established to change the course of liver fibrosis . We have still a lot to learn about the pathogenesis of this disease and what are the pathways to target for therapy. However, during the recent years, there have been big development in identifying some of the cellular and molecular mechanisms involved.

The goal of the present study was to evaluate the potential role of crude extract of *Cystoseira myrica* and *Padina pavonica* against hepatic injury induced by thioacetamide in rats.

MATERIALS AND METHODS

I. Materials

1. Thioacetamide (TAA)

TAA was purchased from Sigma Aldrich. Chemical. Co., (St Louis, Mo, USA) as pure crystals. It was dissolved in saline and freshly prepared prior to each injection.

2. Collecting brown algae

Brown algae were collected from two different locations along the Red Sea coast. For *Cystoseira myrica*, nine Kg was collected from the Napq protected area, South Sinai Governorate, Egypt from the mangroove area at about 0.5 m depth. While, seven Kg of *Padina pavonica* was collected from Nuweiba coast, eastern part of Sinai Peninsula, Egypt from a coral beach at about 1.5 m depth. *Cystoseira myrica* and *Padina pavonica* were identified by Professor Muhammad Hegazi ; Professor of Marine Science, Department of Marine Science, Faculty of Science ,Suez Canal University, Ismailia, Egypt.

3. Pretreatment of seaweeds materials

All samples were washed thoroughly with seawater followed by tap water immediately after collection and were cleaned from any epiphytes as much as possible by using fine brush. About half of the quantity of *Cystoseira myrica* and *Padina pavonica* were frozen at about -20° C until use and the other half was air-dried at room temperature in a darkened room and milled to fine powder.

4. Extracts preparation

The frozen quantity of *Cystoseira myrica* and *Padina pavonica* were defrost, washed in tap water, cut into small pieces and then mixed with 80% methanol, homogenized using electrical blender and extracted three times with 80% methanol each time. Meanwhile, the resulting powder from each air-dried alga was directly extracted three times with 80% methanol each time. At each time, the extract was shaken slowly on a reciprocating shaker overnight at dark. Then, the extracts were filtered using Buchner funnel under suction and each filtrate was concentrated using rotary evaporator at 40°C till it became free of methanol.

5. Experimental animals

Sixty adult male albino rats of Wistar strain (2-3) month-old with body weight between (120-140 g) were obtained from a breeding stock maintained in the Animal House of the National Research Centre, Dokki, Giza, Egypt and acclimatized in a specific area where temperature (25±1ºC) and humidity (55%). Rats were controlled constantly with 12 hours light/dark cycles at National Research Centre, Animal Facility Breeding Colony. Rats were housed with *ad libitum* access to standard pellet diet and tap water. Animals were cared for according to the guidelines for animal experiments.

After acclimatization period (one week), the animals were randomly assigned into six experimental groups (10 rats/ group). The first group was negative control group received normal saline solution intraperitoneally (i.p) twice weekly for 8 weeks. The groups from second to sixth were injected intraperitoneally (i.p) with thioacetamide (SIGMA, USA; dissolved in 0.9% normal saline solution) in a dose of 200 mg/kg b.wt , twice weekly for 8 weeks for induction of liver fibrosis [13]. Then, the second group was left untreated for 8 weeks (positive control). The third and fourth groups were treated orally with *C. myrica* fresh and dry extracts respectively in a dose of 50 mg/kg b. wt. for 8 weeks. While, the fifth and sixth groups were treated orally with *P. pavonica* fresh and dry extracts respectively in a dose of 50 mg/kg b. wt. for 8 weeks [14].

At the end of treatment period, the animals were fasted overnight and the blood samples were withdrawn from the retro-orbital venous plexus of all animals under diethyl ether anesthesia [15]. Each blood sample was divided into two portions, the first one was collected in heparinized tube and centrifuged at 1800 xg for 10min. at 4°C for separation of plasma. Plasma samples were frozen and stored at -20°C for the determination of hydrogen peroxide (H_2O_2) and total antioxidant capacity (TAC) content. While the second portion of each blood sample was collected in dry clean centrifuge tubes and allowed to clot for 30 min. at 25°C. These samples were then centrifuged at 1800 x g for 15 min at 4°C. The top yellow serum layer was pipetted off, without disturbing the white buffy layer, in clean eppendorf tube, frozen and stored at -20°C for the determination of liver enzymes activity (AST, ALT & ALP), triglycerides (TG), cholesterol (Chol), LDL, HDL and tumor necrosis factor alpha (TNF-α) levels. Animals were sacrificed and a midline abdominal incision was performed and the whole liver of each animal was rapidly and carefully excised, thoroughly washed with ice cold isotonic saline, blotted dry and then divided into two portions. The first portion was weighed and homogenized immediately in 50 mM ice cold phosphate buffer (pH 7.4) to give 2% homogenate (w/v) then, the homogenate was centrifuged at 1000 xg for 10 min in cooling centrifuge at 4°C and the supernatant (2%) was used for the determination of hydrogen peroxide ($H_2O_{2)}$ and total antioxidant capacity (TAC). The second portion was fixed in 10% formal saline for twenty four hours for histopathological investigation.

II. Methods

1.Biochemical analysis

Serum AST activity was quantified by kinetic quantitative method using Stanbio kit (Texas USA) according the method described by Wilkinson**[**16**].**Serum ALT activity was estimated by Reitman-Frankel colorimetric method using Quimica Clinica Aplicada S.A. kit (Spain) according to Aebi [17]. Alkaline Phosphatase (ALP) in serum was assayed by kinetic quantitative method using Stanbio Laboratory kit (Texas, USA) following the method of Bowers and Mcomb[18].Triglycerides was determined in serum by quantitative enzymatic colorimetric method using Stanbio kit (Texas, USA) according to the method of Wahlefeld [19].Cholesterol in serum was measured by using kinetic quantitative method by using Reactivos GPL kit (Barcelona, Spain) following the method of Natio[20].The determination of serum HDL cholesterol was done by precipitating method using Reactivos GPL kit (Barcelona, Spain) according to the method of Grove[21].The estimation of serum LDL cholesterol was performed spectrophotometrically using Biosystem kit (Spain) according to the method described by Assman *et al*.[22].Hydrogen peroxide was assayed by colorimetric method using Biodiagnostic kit (Egypt) following the method of Aebi [23]. Total antioxidant capacity was estimated by using Biodiagnostic kit (Egypt) according to the method of Koracevic *et al*.[24].Serum TNF-α concentration was measured by ELISA procedure using kit purchased from Ray Biotech Co., Georgia, USA, according to the method of Brouckaert *et al*.[25].

2.Histopathological investigation

After fixation of the liver samples from different groups in 10% formal saline for twenty four hours, washing was done in tap water, then serial dilutions of alcohol (methanol, ethanol and absolute ethanol) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56^{o}C in hot air oven for twenty four hours . 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 stains [26] for histopathological examination through the electric light microscope.

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 13. Results are expressed as means ± SE of three independent experiments. Statistical significance of difference was determined using analysis of variance (One Way ANOVA). Further statistical analysis for post hoc comparisons was carried out using LSD test. A level of p <0.05 was defined as statistically significant. Percentage of difference representing the percent of variation with respect to the control groups was also calculated.

RESULTS

Biochemical Parameters

The data in **Table (1)** reveal that administration of TAA produced serious attack on liver as indicated by the significant elevation ($p < 0.05$) in each of AST(131.99%), ALT(192.26%) and ALP(75.91%) activity in serum as compared to the negative control group. However, the treatment with *Cystoseira myrica* or *Padina pavonica* fresh or dry extract showed significant reduction ($p < 0.05$) in serum AST(-19.32% and-29.62% for *C. myrica* fresh and dry respectively, -15.15% and -24.7% for *P. pavonica* fresh and dry respectively), ALT (-22.53% and-34.37% for *C. myrica* fresh and dry respectively, -21.2% and -27.98% for *P. pavonica* fresh and dry respectively),and ALP (-15.55% and -20.73% for *C. myrica* fresh and dry respectively, -12.32% and -19.67% for *P. pavonica* fresh and dry respectively) activity relative to the positive control group.

Table (1): Effect of treatment with brown algae extracts on liver functions of rats bearing hepatic injury

Parameters / Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Negative control	56.9 ± 0.8	43.9 ± 2.1	107.5 ± 2.5
Positive control (TAA)	132.0 ± 4.7 [a] (131.99%)	128.3 ± 5.1[a] (192.26%)	189.1 ± 4.3[a] (75.91%)
TAA+ *C. myrica* fresh	106.5± 5.1[b] (-19.32%)	99.4 ± 9.3[b] (-22.53%)	159.7± 5.3[b] (-15.55%)
TAA+ *C. myrica* dry	92.9 ± 4.4[b] (-29.62%)	84.2 ± 4.9[b] (-34.37%)	149.9 ± 2.6[b] (-20.73%)
TAA+*P. pavonica* fresh	112.0 ± 3.5[b] (-15.15%)	101.1 ± 8.9[b] (-21.2%)	165.8 ± 5.7[b] (-12.32%)
TAA+ *P. pavonica* dry	99.4 ± 3.5[b] (-24.7%)	92.4 ± 8.5[b] (-27.98%)	151.9 ± 2.0[b] (-19.67%)

Results are expressed as means ±SD for 10 rats / g roup.
a: significant change compared with the negative control group.
b: significant change compared with the positive control group.
(%): percent of difference with respect to corresponding control value.

The findings in **Table (2)** show significant increase ($p < 0.05$) in serum TG (30.01%), Chol (40.24%) and LDL (57.67%) levels parallel by significant decrease ($p < 0.05$) in serum HDL (-23.88%) level in TAA intoxicated group in comparison with the negative control group.

Table (2): Effect of treatment with brown algae extracts on lipid profile of rats bearing hepatic injury

Parameters / Group	TG (mg/dL)	Chol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Negative control	75.3 ± 6.5	58.4 ± 5.8	26.8 ± 2.4	16.3 ± 0.9
Positive control (TAA)	97.9± 7.1[a] (30.01%)	81.9 ± 6.3[a] (40.24%)	20.4 ± 0.5[a] (-23.88%)	25.7 ± 2.0[a] (57.67%)
TAA+*C .myrica* fresh	88.1 ± 8.1 (-10.01%)	67.3 ± 3.2[b] (-17.83%)	23.6 ± 0.5 (15.69%)	20.2 ± 0.7[b] (-21.4%)
TAA+*C. myrica* dry	80.5 ± 7.3 (-17.77%)	63.1 ± 3.5[b] (-22.95%)	24.9 ± 1.6 [b] (22.06%)	18.8 ± 1.8[b] (-26.85%)
TAA+*P. pavonica* fresh	90.7 ± 4.3 (-7.35%)	74.4 ± 6.1 (-9.16%)	22.4 ± 1.2 (9.8%)	22.9 ± 1.2 (-10.89%)
TAA+*P. pavonica* dry	84.4 ± 8.1 (-13.79%)	64.9 ± 2.9 [b] (-20.76%)	24.2 ± 1.4 (18.63%)	19.8 ± 1.4[b] (-22.96%)

Results are expressed as means ±SD for 10 rats / g roup.
a: significant change compared with the negative control group.
b: significant change compared with the positive control group.
%: percent of difference with respect to corresponding control value.

The treated groups with *C. myrica* or *P. pavonica* fresh or dry extracts revealed insignificant decrease ($p > 0.05$) in serum TG level (-10.01% and-17.77% for *C. myrica* fresh and dry respectively; -7.35% and -13.79% for *P. pavonica* fresh and dry respectively) as compared to the positive control group. Significant decrease ($p < 0.05$) in Chol level was observed in the group treated with *C. myrica* fresh (-17.83%) and dry(-22.95%) as well as *P. pavonica* dry (-20.76%) extracts. Meanwhile, insignificant ($p > 0.05$) decrease in serum cholesterol level was recorded in the group treated with *P. pavonica* fresh (-9.16%) extract with respect to the positive control group. Similarly, serum LDL level showed significant reduction ($p < 0.05$) in the group treated with *C. myrica* fresh (-21.4%) and dry(-26.85%) as

well as *P. pavonica* dry (-22.96%) extracts but it revealed insignificant reduction ($p > 0.05$) in the group treated with *P. pavonica* fresh (-10.89%) extract when compared with the positive control group. Regarding serum HDL, it showed significant ($p < 0.05$) increase in the treated group with *C. myrica* dry (22.06%) extract while, it revealed insignificant ($p > 0.05$) increase in the treated group with *C. myrica* fresh (15.69%), *P. pavonica* fresh (9.8%) or dry (18.63%) extract as compared to the positive control group **(Table 2)**

The results in **Table (3)** reveal significant ($p < 0.05$) increase in plasma H_2O_2 level in TAA-intoxicated group (78.72%) as compared to the negative control group. But as for TAC, it showed significant ($p < 0.05$) decrease in TAA-intoxicated group (-45.21%) as compared to the negative control group. Administration of *C. myrica* or *P. pavonica* dry extract resulted in significant ($p < 0.05$) decrease in plasma H_2O_2 level (-19.64% and -17.26% respectively). Meanwhile, the administration of *C. myrica* or *P. pavonica* fresh extract led to insignificant ($p > 0.05$) decrease in plasma H_2O_2 level (-13.69% and -10.71% respectively) relative to the positive control group. On the other side, significant ($p < 0.05$) increase in plasma TAC was detected in the treated groups with either *C. myrica* fresh (38.75%), *C. myrica* dry (60%) or *P. pavonica* dry (47.5%) extract with respect to the positive control group. Of note, the treatment with *P. pavonica* fresh extract caused insignificant ($p > 0.05$) increase in plasma TAC(21.25%) comparing with the positive control group **(Table 3)**

Table (3): Effect of treatment with brown algae extracts on plasma oxidant / antioxidant status of rats bearing hepatic injury

Parameters / Groups	H_2O_2 (µM / mL)	TAC (mM / L)
Negative control	9.4 ± 0.94	1.46 ± 0.05
Positive control (TAA)	16.8 ± 1.0[a] (78.72%)	0.8 ± 1.001[a] (-45.21%)
TAA+ *C. myrica* fresh	14.5 ± 0.9 (-13.69%)	1.11± 0.06 [b] (38.75%)
TAA+ *C. myrica* dry	13.5 ± 1.1[b] (-19.64%)	1.28 ± 0.06 [b] (60%)
TAA+ *P. pavonica* fresh	15.0 ± 1.1 (-10.71%)	0.97 ± 0.06 (21.25%)
TAA+ *P. pavonica* dry	13.9 ± 0.97[b] (-17.26%)	1.18 ± 0.07 [b] (47.5%)

Results are expressed as means ±SD for 10 rats / group.
a: significant change compared with the negative control group.
b: significant change compared with the positive control group.
%: percent of difference with respect to corresponding control value.

Table (4): Effect of treatment with brown algae extracts on hepatic oxidant / antioxidant status of rats bearing hepatic injury

Parameters / Groups	H_2O_2 (µM/g.tissue)	TAC (mM/g.tissue)
Negative control	105.1 ± 6.8	44.4 ± 2.2
Positive control (TAA)	175.1 ± 12.6[a] (39.98%)	22.8 ± 0.08[a] (-48.65%)
TAA+ *C. myrica* fresh	143.7 ± 5.6[b] (-17.93%)	35.3 ± 0.7[b] (54.82%)
TAA+ *C. myrica* dry	139.9 ± 5.7[b] (-20.1%)	37.0 ± 0.4[b] (62.28%)
TAA+ *P. pavonica* fresh	151.9 ± 11.9 (-13.25%)	32.7 ± 1.6[b] (43.42%)
TAA+ *P. pavonica* dry	140.9 ± 9.8[b] (-19.53%)	36.0 ± 1.2[b] (57.89%)

Results are expressed as means ±SD for 10 rats / group.
a: significant change compared with the negative control group.
b: significant change compared with the positive control group.
%: percent of difference with respect to corresponding control value.

The data in **Table (4)** reveals that the intoxicated TAA group displayed significant ($p < 0.05$) elevation in hepatic H_2O_2 (39.98%) level as compared to the negative control group. On the contrary, it showed significant ($p<0.05$) reduction in hepatic TAC (-48.65%) when compared with the negative control group.The groups that received fresh or dry extract of *C. myrica* or dry extract of *P. pavonica* exhibited significant ($p<0.05$) reduction in hepatic H_2O_2 levels(-17.93%, -20.1% and -19.53% respectively). Treatment with *P. pavonica* fresh extract induced insignificant ($p > 0.05$) reduction in hepatic H_2O_2 level(-13.25%) level relative to the positive control group. On the other side

the groups that received *C. myrica* fresh or dry extract as well as *P. pavonica* fresh or dry extract exerted significant ($p<0.05$) rise in hepatic TAC (54.82%, 62.28%, 43.42% and 57.89% for *C. myrica* fresh, *C. myrica* dry, *P. pavonica* fresh, *P. pavonica* dry respectively) with respect to the positive control group.

Histopathological investigation

Histopathological investigation of liver tissue sections of rats in the different studied groups.

1. Group of rats kept as negative control

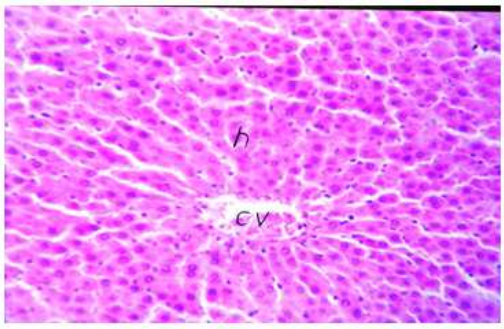

Fig 1

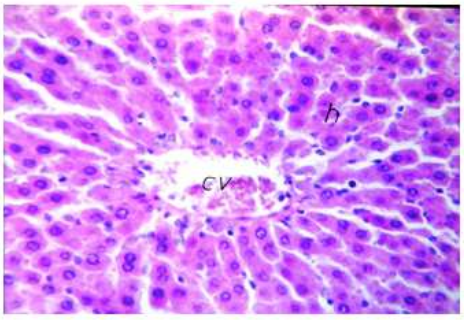

Fig 2

Fig (1) Photomicrograph of liver tissue section of rat in the negative control group showing normal histological structure of the central vein (cv) and surrounding hepatocytes (h) (H&E X200)

Fig (2) Photomicrograph of liver tissue section of rat in the negative control group showing the magnification of Fig (1) (H&E X400)

2. Group of rats kept as positive control

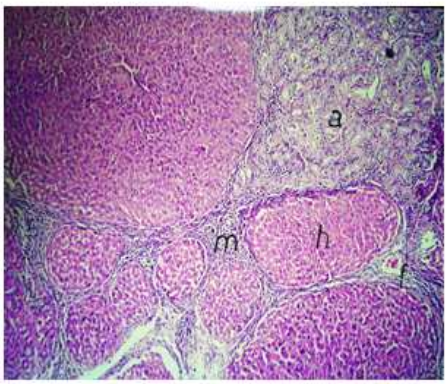

Fig 3

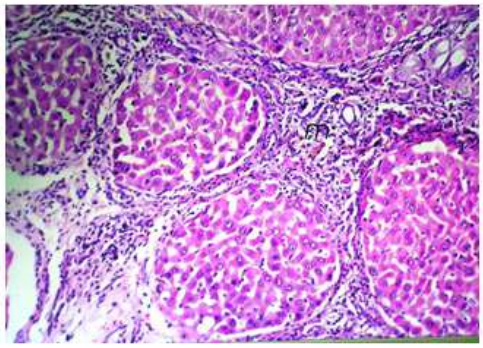

Fig 4

Fig (3) Photomicrograph of liver tissue section of rat in the positive control group showing fibrosis with inflammatory cells infiltration (m) with acini formation (a) dividing the parenchyma into lobules and nodules (H&E X200)

Fig (4) Photomicrograph of liver tissue section of rat in the positive control group showing the magnification of Fig (4) to identify the nodular formation (H&E X400)

Table (5): Effect of treatment with brown algae extracts on serum TNF-α of rats bearing hepatic injury

Parameters / Groups	TNF-α (pg/mL)
Negative control	77.14± 6.2
Positive control (TAA)	104.2 ± 5.4[a](35.08%)
TAA+ *C. myrica* fresh	87.5 ± 5.1[b](-16.03%)
TAA+ *C. myrica* dry	82.5 ± 4.8[b](-20.83%)
TAA+ *P. pavonica* fresh	94.0 ± 3.3(-9.79%)
TAA+ *P. pavonica* dry	85.9 ± 3.2[b](-17.56%)

Results are expressed as means ± SD for 10 rats / group.
a: significant change compared with the negative control group.
b: significant change compared with the positive control group.
%: percent of difference with respect to corresponding control value.

The results in **Table (5**) demonstrate significant ($p < 0.05$) increase in serum TNF-α level (35.08%) in TAA-intoxicated group against the negative control group. Meanwhile the groups that received *C. myrica* fresh or dry extract or *P. pavonica* dry extract revealed significant ($p<0.05$) decrease in serum TNF-α level (-16.03%, -20.83% and -17.56% respectively). Noteworthy, the group that received *P. pavonica* fresh extract showed insignificant ($p > 0.05$) decrease in serum TNF-α level (-9.79%) when compared with the positive control group.

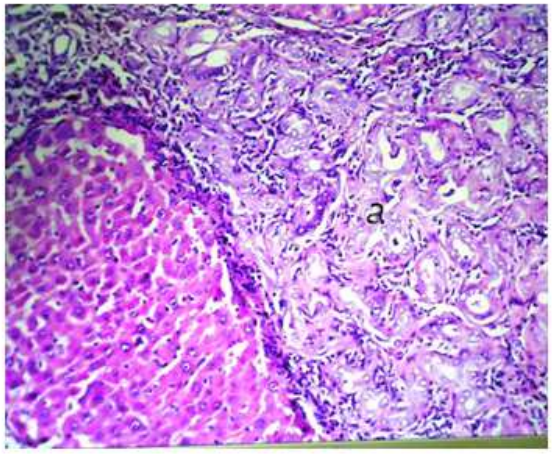

Fig (5) Photomicrograph of liver tissue section of rat in the positive control group showing formation of acini of anaplastic hepatocytes (a) in high magnification (H&E X400)

3.Group of rats treated with *Cystoseira myrica* fresh extract

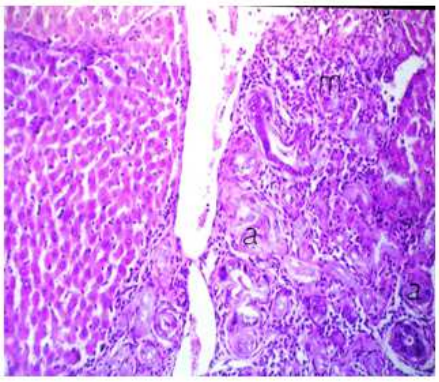

Fig 6

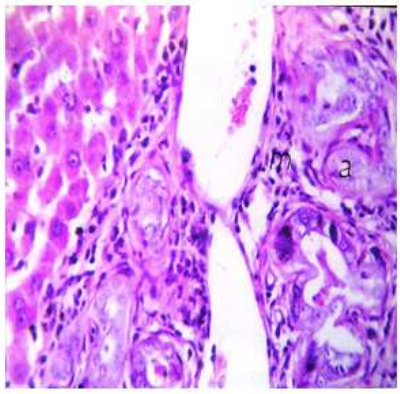

Fig 7

Fig(6) Photomicrograph of liver tissue section of rat treated with *C. myrica* fresh extract showing focal area of the hepatic parenchyma with inflammatory cells infiltration (m) in between hepatocytes forming acini like glandular structure (a) (H&E X200)

Fig (7) Photomicrograph of liver tissue section of rat treated with *C. myrica* fresh extract showing the magnification of Fig (6) to identify the fibrosis and inflammatory cells infiltration (m) in between hepatocytes forming acini like glandular structure (a) (H& X400)

4. Group of rats treated by *Cystoseira myrica* dry extract

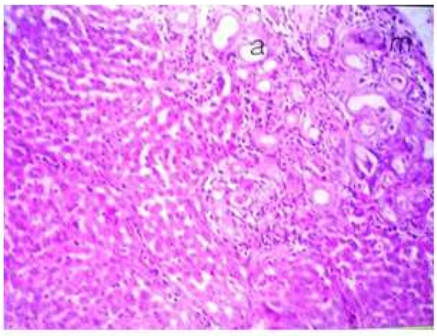

Fig 8

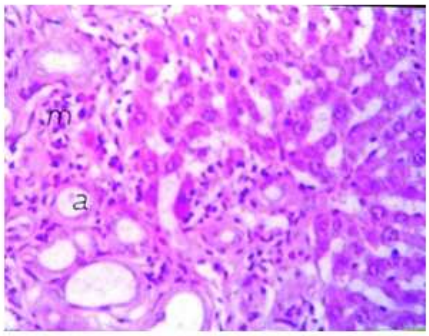

Fig 9

Fig. (8) Photomicrograph of liver tissue section of rat treated with *C. myrica* dry extract showing focal area of hepatic parenchyma with inflammatory cells infiltration (m) in between the hepatocytes acini formation (a) (H&E X200)

Fig. (9) Photomicrograph of liver tissue section of rat treated with *C. myrica* dry extract showing the magnification of Fig (8) (H&E X400)

5.Group of rats treated with *Padina pavonica* fresh extract

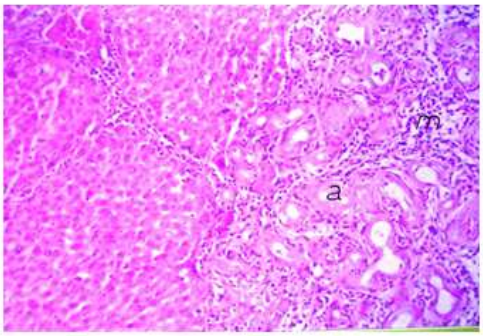

Fig 10

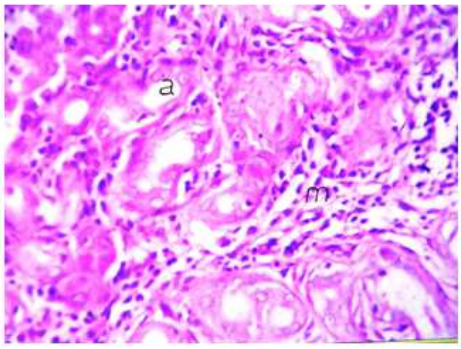

Fig 11

Fig.(10) Photomicrograph of liver tissue section of rat treated with *P. pavonica* fresh extract showing focal area of hepatocytes forming acini like glandular structure (a) with inflammatory cells infiltration (m) (H&E X200)

Fig.(11) Photomicrograph of liver tissue section of rat treated with *P. pavonica* fresh extract showing the magnification of Fig (10) (H&E X400)

6.Group of rats treated with *Padina pavonica* dry extract

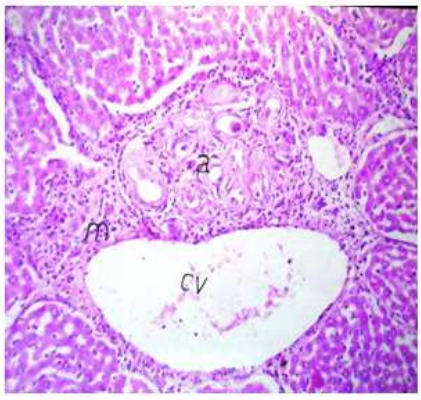

Fig 12

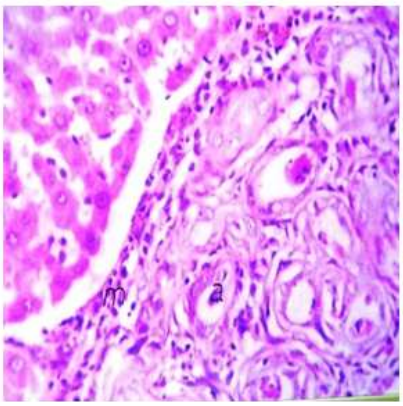

Fig 13

Fig. (12) Photomicrograph of liver tissue section of rat treated with *P. pavonica* dry extract showing focal area of hepatocytes forming acini (a) with inflammatory cells infiltration (m) (H&E X200)

Fig.(13) Photomicrograph of liver tissue section of rat treated with *P. pavonica* dry extract showing the magnification of Fig. (12) (H&E X400)

DISCUSSION

The current study was constructed to explore the efficacy of two species of brown algae (*Cystoseira myrica* and *Padina pavonica*) against TAA-induced hepatic injury in rats. This goal was achieved through assessment of liver functions, analysis of lipid panel, evaluation of oxidant / antioxidant status, estimation of inflammatory intermediator as well as examination of the histological features of liver tissue.

The present results demonstrate that TAA administration caused significant increase in the activity of liver enzymes (AST, ALT and ALP) in serum. This stems from the leakage of these enzymes from liver cells into the circulation as a result of hepatic tissue damage [27]**.** This result could be explained by the generation of free radicals after TAA intoxication which could affect hepatic cellular membrane permeability leading to the elevation of the circulating values of these enzymes[28]**.** These findings are in agreement with the previous studies which showed that these enzymes were statistically increased in serum of experimental animals administered TAA[29,30]**.**

In the present study, TAA significantly increased serum cholesterol, triglycerides and LDL levels. Also, there was significant decline in the level of HDL due to TAA administration. These data indicate the onset of disturbances in protein, carbohydrate and lipid metabolism as consequence of TAA intoxication[31,32].

The current results indicate that the administration of TAA experienced significant increase in both plasma and hepatic H_2O_2 level paralleled by significant decrease in plasma and hepatic TAC. TAA hepatotoxicity results from its metabolic conversion to free radical products: TAA S-oxide and TAA S-dioxide produced during cytochrome-P450-mediated oxidation of TAA[33]. These metabolites attack microsomal lipids leading to their peroxidation and production of reactive oxygen species (ROS), such as the H_2O_2, super oxide anion O_2^-, and the hydroxyl radical. ROS could affect the antioxidant defense mechanisms and decrease the activity of the antioxidant enzymes.

The present findings demonstrate that TAA intoxication increased serum TNF-α level. This result could be attributed to the oxidative stress and the induction of excess ROS which in turn activates NF-κB[34] and once NF-κB is activated, a consequent increase in TNF-α serum level occurs [35].

The obtained results show that the administration of TAA-intoxicated rats with *C. myrica* or *P. pavonica* dry or fresh extract elicited significant reduction in serum AST, ALT and ALP activity. This could be achieved by the ability of the active constituents of the crude methanolic extracts of these seaweeds to preserve the structural integrity of plasma cellular membrane of the hepatocytes to protect it against breakage by the reactive metabolites produced from exposure to TAA. This finding is in accordance with Vazquez-Freire *et al.* [36] who stated that the hepatoprotective effect of seaweeds could be attributed to their free radical scavenging activity. This property of seaweeds is mostly related to their phenolic contents (phlorotanins)[37,38].

The present results reveal that the studied brown algae extracts improved lipid profile in TAA-intoxicated rats. This effect has been shown from the significant reduction in serum cholesterol, triglycerides and LDL levels. Jimenez-Escrig and Goñi [39] reported that marine macroalgae contain fucosterol, desmosterol, sargasterol, stigmasterol, beta-sitosterol and ergosterol. These compounds could reduce serum cholesterol by: 1) interfering with the endogenous synthesis of cholesterol and 2) competing with the cholesterol for the absorption sites at the small intestine level. The hypocholesterolemic effect of these polysaccharides is attributed to the formation of viscous systems in the small intestine that diminish the speed of passage of nutrients such as glucose and lipids to the blood. The colloidal particles formed in the intestine retain cholesterol and biliary acids, forming a colloid of ionic type, that is later excreted in the feces[40]**.** Moreover, brown algae are an important source of minerals such as copper (4.7 mg 100g-1) [41], in the organic form with great bioavailability[42]**.** Copper can cause an indirect regulation of cholesterol biosynthesis through the drop in the reduced glutathione (GSH) and the rise in the oxidized glutathione (GSSG). The later decreases the activity of 3-hydroxy-3-methylglutaryl-coenzyme A (HMGCoA). This makes the reduction of carbon flow through the mevalonate pathway with consequent inhibition of cholesterol synthesis[43]. Furthermore, brown algae are characterized by having high iodine content [41]**,** that has hypocholesterolemic properties [44]. Besides, Dianzani [45] stated that the sulphur compounds in the brown algae extracts are responsible for reducing the excessive accumulation of triglycerides.

It is observed from the present study that brown algae extracts significantly lowered serum LDL level. This comes in line with the study of Raghavendran *et al.* [46] which indicated that phenolic compounds had the ability to form

phenoxyl radicals in the presence of peroxidases preventing LDL peroxidation and retarding its accumulation. Xue *et al*. [47] mentioned that fucoidans in brown algae possessed powerful activity in scavenging free radicals and inhibiting LDL oxidation[48].

HDL is a free radical scavenger and prevents peroxidation of beta lipoproteins[49]. In the current study, rats treated with brown algae extracts showed great improvement in serum level of HDL, which might be due to the ability of brown algae active constituents to hasten the decomposition of free radical species[46].

Hydrogen peroxide (H_2O_2) is a reactive nonradical compound that can penetrate biological membranes and converted into more reactive species such as singlet oxygen and hydroxyl radicals. The present results indicate that brown algae extracts decreased both plasma and hepatic H_2O_2 levels in TAA intoxicated rats. Heo *et al.* [50] reported that brown algae (*Ecklonia cava*) extract could strongly scavenge H_2O_2 in *vitro*. This extract showed good inhibitory effects against DNA damage not only at high concentrations but also at low concentrations. DNA damage is known to be one of the most sensitive biological markers for evaluating oxidative stress representing the imbalance between free radical generation and efficiencies of the antioxidant system [51,52]. The study of Peerapornpisal *et al.* [53] indicated that the aqueous polysaccharides from brown algae were able to reduce free radicals as it had a strong free radical scavenging ability particularly for hydroxyl radical.

In the current study brown algae extracts significantly increased both plasma and hepatic TAC in TAA intoxicated rats. Polyphenols are known to be effective antioxidants as they easily transfer a hydrogen atom to lipid peroxyl radicals to form the aryloxyl, which is incapable of acting as a chain carrier or couples with another radical and thereby quenches the radical process[54]. Federica *et al.* [55] observed that the radical scavenging activity increased in a group of brown algae with increased phenolic content. This conversation agrees with Heo *et al.* [56] who proved the positive correlation between DPPH radical scavenging activity and total polyphenolics in *Ecklonia cava* enzymatic extracts. Moreover, Athukorala *et al.* and Siriwardhana *et al.* [57,58] reported that antioxidant compounds from seaweed extracts have better ROS scavenging activity and inhibitory effect of lipid peroxidation than commercial antioxidants. By the same way, the study of Peerapornpisal *et al.* [53] demonstrated the antioxidant activity of brown algae which has been attributed to their ability to reduce oxygen free radicals *via* decreasing ferrous ion in the assay system. Taken together, these properties of brown algae enable them to enhance TAC of rats subjected to TAA intoxication.

The results of the present work demonstrate that administration of brown algae extracts in TAA-intoxicated rats reduced TNF-α serum level. This result indicates the antiinflammatory properties of these extracts. It has been reported that the secretion profiles of proinflammatory cytokines, including IL-1β, IL-6, TNF-α, and NO were significantly reduced upon the treatment with sulphated polysaccharides from brown algae in *vitro*. This was proved by the study of Hwang *et al.* [59] which explored that sulphated polysaccharides from brown seaweed *Sargassum hemiphyllum* have antiinflammatory effect due to their ability to downregulate NF-KB in the nucleus.

The secondary metabolites from the genus *Cystoseira*n are polysaccharides, sterols, lipids, terpens and many diterpenoids which have been isolated as the linear diterpenes or acyclic and cyclic meroditerpenoids[60]**.** These metabolites have been suggested to act synergistically to exert many pharmacological activities including antiinflammatory activity[61]**.**

Similarly, the antiinflammatory activity of *Padina pavonica* extract is possibly ascribed to its active components; sterols, fatty acids, aromatic esters, terpenoids, benzyl alcohol and benzaldehyde [62] by which it could effectively decrease the production of proinflammatory cytokines including TNF-α.

In conclusion, the present results shed light on the beneficial effects of *Cystoseira myrica* and *Padina pavonica* extracts in management of hepatotoxicity induced by thioacetamide in rats. The potential role of the selected algae extracts in regression of hepatic injury was likely attributed to the hepatoprotective, hypolipidemic, antioxidant and antiinflammatory properties of their phytochemicals. In view of the present data, it seems that brown algae may be a good source of functional ingredients that can be used for alleviating liver diseases. However, further studies including detailed toxicity analysis are needed to determine the usefulness of these macroalgae in mitigating such diseases. Of note, insights into the superior effect of dry algae extract over the fresh one might be ascribed to concentrating the bioactive ingredients due to air-drying. Yet it is better to be stored in the dried form for a long time without any oxidative deterioration.

REFERENCES

[1] D Govindasamy, N Ford, K Kranzer, *AIDS*. **2012**, 26, 2059-2067.
[2]B Kolonjnattan, M Demarty, S Mabeau, *Int. J. Biol. Macromol*. **2009**, 8, 380–386.
[3]P Rajasulochana, R D hamotharan, P Krishnamoorthy S Murugesan , *J. Am. Sci*.**2009**, 5, 20–25.
[4]R Matanjun, Z Dubinsky ,E Kishimoto, K Masaki, Y Masuda, T Takeuchi, M Chihara, Y Yamamoto, E Niki, I Karube, *J. Appl. Phycol*. **2002**, 9, 29–35.
[5]JE Painter,TF Slater, RL Wilson, *Life Sci*. **1983**, 23, 2617–2620.
[6]RE Lee, Phycology, Cambridge University Press.**2008**,560, 4th ed.
[7]L Chevolot, B Mulloy, J Ratiskol, A Foucault, S Colliec-Jouault, *Carbohyd. Res.***2001**, 330, 529–535.
[8]KD Thomps, C Drager, *Res*.**2004**, 18,551-555.
[9]H Maruyamaa, H Tamauchib, M Iizuka, T Nakano, *Planta Med*. **2006**, 72, 1415-1417.
[10]A Cumashi, NA Ushakova, ME Preobrazhenskaya, A D'Incecco, A Piccoli, L Tatoni, N Tinari, GE Morozevich, AE Berman, MI Bilan, AI Usov, NEUstyuzhanina, AA Grachev, CJ Sanderson, M Kelly, GH Rabinovich, S Lacobelli, NE Nifantiev, *Glycobiology*. **2007**, 17, 541-552.
[11]M Jung So, B Kyung, S Hee, E Ju, *J. Food Sci Nutr*. **2007**, 12,191-195.
[12]C Griffiths, C Rooney, A Brock, *Health Statistics Quarterly*. **2005**, 28, 6-17.
[13]R Bruck, H Shirin, H Aeed, Z Matas, A Hochman, M Pires, Y Avni, *J. Hepatol*. **2001**, 35, 457-64.
[14] S Hayashi, A Itoh, K Isoda, M Kondoh, M Kawase, K Yagi , *Eur. J. Pharmacol*.**2008**, 580, 380-384.
[15]S Schermer**,** The Blood Morphology of Laboratory Animals, F.A. Davi, Philadelphia, **1967,**(3rd ed.),p42.
[16]JH Wilkinson. In: J H Wilkinson Chicago (Ed.),The Principles and Practice of Diagnostic Enzymology,Year Book Medical Publishers, Inc,**1976,**p250.
[17]M Aebi. In: HU Bergmeyer (ed), Methods of enzymatic analysis, Verlag Chemie-Academic Press ,New York,**1974**, (Vol. 2), 735-739.
[18]GN Bowers, RB McComb, *Clin Chem*. **1966**, 12,70-89.
[19] AW Wahlefeld. In: HU Bergmeryer (ed), Methods of Enzymatic Analysis, Academic Press, New York,1974, (Vol.5),1831-1835.
[20]HK Natio. In:A Kaplan et al (ed), Clin Chem, The C. V. Mosby Co, St Louis, Toronto, Princeton, 1984, 1194-11206.
[21]TH Grove, *Clin. Chem*. **1979**, 25, 560-564.
[22]G Assman, HU Jabs, U Kohnert, W Nolte, H Schriewer, *Clin.Chim.Acta*.**1984**, 140,77-83.
[23]H Aebi ,*Methods Enzymol*. **1984**, 105(C),121-126.
[24]D Koracevic, G Koracevic, V Djordjevic, S Andrejevic ,V Cosic, *J. Clin. Pathol*.**2001**, 54, 356-61.
[25]P Brouckaert ,C Libert ,B Everaerdt ,N Takahashi ,A Cauwels , W Fiers, *Immunobiol*.**1993**, 187, 317-29.
[26]JD Banchroft, A Stevens, DR Turner, Theory and practice of histological techniques, Churchill Livingstone, New York, London, San Francisco, Tokyo,1996,(4th ed.),p21.
[27]SS Alkiyumi, MA Abdullah, AS Alrashdi, SM Salama, SI Abdelwahab, AA Hadi , *Molecules*. **2012**,17, 6146-6155.
[28]M Amer, S Areida, *J. Egypt Ger*. Soc. Zool. **2004**, 44(A) , 199-219.
[29]H Shapiro, M Ashkenazi, N Weizman, M Shahmurov, H Aeed, R Bruck, *J Gastroenterol Hepatol*. **2006**, 21, 358–366.
[30]MA Alshawsh, MA Abdulla, S Ismail, ZA Amin, *Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine*.**2011**,10,1155.
[31]BK Tripathi, S Srivastava, R Rastogi, D Raina, VJ Ram, AK Srivastava, *Acta Pharmaceutica*. **2003**, 53, 91–100.
[32]I Swapna, KV SathyaSaiKumar, Ch R K Murthy, 1 A Dutta-Gupta, B Senthilkumaran, *Journal of Neurochemistry*. **2006**, 98,1899–1907.
[33]E Chieli, G Malvaidi, *Toxicology*.**1984**, 31, 41-52.
[34]E Ho, TM Bray, *Proc. Soc. Exp. Biol. Med*. **1999**, 222, 205-213.
[35]IA Leclercq, GC Farrell, C Sempoux, AD Pena, Y Horsmans, *J. Hepatology*. **2004**, 41, 926-934.
[36]MJ Vazquez-Freire ,M Lamela, J Anca, A Gato, JM Calleja, Actes du 2e Colloque Européen Ethopharmacologie et del a 1l e Conference internationale, Ethomedecine, Heidelberg.**1993,** 24-27.
[37]CK Wong, CE Ooi, O Ang, *Hydrobiologia*. **2004**, 512, 267–270.
[38]DR Karthikeyan, T Manivasagam, P Subramaniam, ST Somasundaram, P Anantharaman ,T Balasubramainan, *Seaweed. Res*. **2007**, 30, 157–163.
[39]A Jimenez-Escrig, I Goni, *Arch. Latinoam Nutr*. **1999**, 49, 114-120.

[40]LN Panlasigui, OQ Baello, JMDimatangal, BD Dumelod, *Asia-Pacific J. Clin. Nutr*.**2003**, 12, 209.
[41]Y Yuan. In: C Barrow, F Shahidi (ed), Marine nutraceuticals and functional foods, CRC Press, Boca Raton, Florida, USA, 2008, p259.
[42]VJ Chapman , DJ Chapman.In: Seaweeds and Their Uses, Chapman and Hall, London, New York,1980, (3rd edition.),62–97.
[43]HF Gilbert ,MD Stewart, *J. Biol. Chem*. **1981**, 256,1782.
[44]K Kaji, Y Seyama, S Yamashita, *Nippon Yakurigaku Zasshi*. **1984**, 4, 325.
[45]MU Dianzani. In: TF Slatter (ed), Biochemical Mechanisms of Liver Injury, Academic Press,New York,**1978**.
[46]HRB Raghavendran, A Sathivel, T Devaki, *Molecular and Cellular Biochemistry*.**2005**, 276, 89–96.
[47]CH Xue, Y Fang, H Lin, L Chen, ZJ Li, D Deng, CX Lu , *J. Appl. Phycol*. **2001**, 13, 67–70.
[48]JH Kang, G Tsuyoshi, IS Han, T Kawada, YM Kim ,R Yu , *Obesity*. **2003**, 18,780-787.
[49]R Chander, NK Kapoor, *Biochem Pharmacol*. **1990**, 40, 1663–1665.
[50]SJ Heo, PT Park, EJ Park, SK Kim, YJ Jeon, *Eur Food Res Technol*. **2005**, 221, 41–47.
[51]F Kassie, W Pqrzefall, S Knasmuller, Res. **2000**, 463,13– 31.
[52]ET Donelly, *Mutagenesis*. **1999**, 14,505–512.
[53]Y Peerapornpisal, D Amornlerdpison, U Jamjai, T Taesotikul, Y Pongpaibul, M Nualchareon, D Kanjanapothi ,Chiang Mai, *J. Sci*. **2010**, 37, 507-516.
[54]G Ruberto, MT Baratta, DM Biondi, V Amico, *J. Appl. Physiol*. **2001**, 13,403–407.
[55]P Federica, B Stefania, M Lara, M Michele, S Fabrizia, *J. Pharm Biomed*. **2004**, 35,289–301.
[56]SJ Heo, KW Lee, CB Song, YJ Jeon, *Algae*. **2003**, 18, 71-81.
[57]A Athukorala, KW Lee, CB Song, CB Ahn, TS Shin, YJ Cha, F Shahidi, YJ Jeon, Asian crisis, *J. Food Lipids*. **2003**, 10,251–265.
[58]N Siriwardhana, KW Lee, SH Kim, JW Ha, YJ Jeon, *Food Sci Tech Int*. **2003**, 9: 339–346.
[59]DF Hwang, CH Wu, SY Gau,SYChein, PA Hwang, *J. Mar. Sci. and Tec*. **2011**, 18, 41-46.
[60]G Culioli, A Ortalo-Magne ,M Daoudi, H Thomas-Guuyon, R Valls, L Piovetti, *Phytochemistry*. **2004**, 65, 2063-2069.
[61]T Geetha, P Varalakshmi, *J. Ethnopharmacol*. **2001**, 76, 77-80.
[62]Z Kamenarska, FN Yalcın, T Ersöz, I C¸alis, K Stefanova, S Popov, *Verlag der Zeitschriftfür Natur for schung, Tübingen*. **2002**,57c, 584-590.