

# Antibacterial and anti-hyperlipidemic activities of the brown alga *Hormophysa cuneiformis* from Ad Dammam Seashore

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## ABSTRACT

A laboratory experiment was conducted to test antibacterial activity of ethanol extract of the brown alga *Hormophysa cuneiformis* against three types of Gram- positive and three of Gram-negative bacteria, where extracts of this alga proved efficient activity against these pathogenic bacteria species ranged between medium and high suppression action. Also, we evaluated the effects of *H. cuneiformis* ethanolic extract and its major constituents on the serum lipid and protein levels of hyperlipidemic rats induced by the administration of a high-cholesterol diet. Results revealed the efficiency of *H. cuneiformis* extract in the decreasing the levels of the total cholesterol (TC), triglycerides (TG), low-density lipoproteins (LDL-C), blood urea nitrogen (BUN) and creatinine caused by alcohol. On the other hand, the treatment by alga extract exhibit significant increase in the high-density lipoproteins (HDL-C) (beneficial), total protein, albumin and globulins. The algal treatments also masking the lethal effects caused by harmful alcohol from raising the rate of enzymes ALT, AST, which returned to normal state in the groups treated by alga extract. Also, the histopathological examination of the ethanol treated groups of rate revealed degeneration and necrotic changes as compared to the administrated rate groups with alga extract which showed marked amelioration of the severity of these changes.

## INTRODUCTION

The necessity of compounds with antioxidant activity is increasing as it is realized that the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been linked in the pathogenesis of several human diseases such as atherosclerosis, diabetes mellitus, chronic inflammation, neurodegenerative disorders and certain types of cancer (Collier *et al.*, 1990, Boynes 1991). Oxygen free radicals disintegrate DNA, destroy cell membranes, and create havoc among cell's basic enzymatic metabolic processes (Kerr *et al.*, 1991). Among the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, diabetes mellitus, cancer, Parkinson's disease and immune dysfunction and is involved in aging (Halliwell, 2000; Metodiewa and Koska, 2000; Young and Woodside, 2001). The formation of cancer cell in human body can be directly induced by free radicals.

Furthermore, ionizing radiation, which causes free radicals, is well documented as a carcinogen. Radicals which have one or more unpaired electrons are produced in normal or pathological cell metabolism. Free Reactive oxygen species (ROS) react easily with free radicals to become radicals themselves. ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals ( $O_2^-$ ) and hydroxyl radicals ( $OH^-$ ), as well as non-free radical species ( $H_2O_2$ ) and the singled oxygen ( $O_2$ ). The antioxidant activity of these compounds are mainly attributed to scavenging activity against superoxide and hydroxyl radicals, chelating ability, quenching singlet and triplet oxygen, and reducing power (Ruberto *et al.*, 2001, Athukorala *et al.*, 2006). It is important to develop, identify and utilize new source of safe and effective antioxidants of natural origin (Li *et al.*, 2007, Qi *et al.*, 2005). There are several synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), Propylgallate (PG) and butylated hydroxyquinone (TBHQ) are commercially available and currently used. However, the use of these synthetic antioxidants for food or medicine components has been restricted by the toxicity and safety that can lead to the problems of the potential health in human.

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Due to the reasons, many researchers have tried to find the more effective oxidation inhibitors that may be used as antioxidants for food or medicine compositions without the side effects for the past several years. So many researchers have paid attention to many kinds of natural antioxidants that can be used without toxicity in human (Capasso 2012). Marine macroalgae are rich sources of several compounds with biological effects including antioxidant activities (Ibraheem *et al.*, 2012).

Marine algae have been used as a novel food with potential nutritional benefits and in industry and medicine for various purposes (Santoso *et al.*, 2004). Recently, aquatic habitats have increasing been shown to provide a rich source of natural bioactive compounds with hypocholesterolemia, antinflammatory, antiviral, antineoplastic, antimicrobial and hypertensive properties. According to their chemical structure, most of isolated compounds belong to sulfated polysaccharides, phenolics, terpenoids, lactons, sterol and fatty acids (Qi *et al.*, 2005, McDermid and Stuercke, 2003, Duan *et al.*, 2006).

On the other hand, the antioxidant activity is one of the most important active in marine bioactive substances and lots of algal and algae-derived compounds exhibited potent antioxidant such as carotenoids, phenolics, terpenoids and sulphated polysaccharides. The antioxidant activity of these compounds are mainly attributed to scavenging activity against superoxide and hydroxyl radicals, chelating ability, quenching singlet and triplet oxygen and reducing power (Ruberto *et al.*, 2001, Athukorala *et al.*, 2006).

In a continuation of the search for algae-derived antihypercholesterolemic and hypolipidemic agents, we evaluated the potential of the brown alga *Hypophysea cuneiformis* as anti-hyperlipidemic agents in rats.

## MATERIAL AND METHODS

### Antibacterial activity of the studied algae

#### *Extraction of selected algal species*

Twenty five grams of the alga dry weight were soaked in 250 ml of ethanol for 24 hours. The waited grams were homogenized in a blender with solvent at room temperature, then filtration and concentration under reduced pressure by using rotary evaporator.

#### Test microorganisms

Three gram-positive bacteria *Staphylococcus aureus* (ATCC29213), Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 12498 and *Enterococcus faecalis* ATCC 29212 and three gram-negative bacteria *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC27738 were used for this test. The bacteria strains were obtained from the Riyadh military Hospital. The bacterial stock cultures were maintained at 4°C.

### Evaluation of antimicrobial Activity

The bacterial strains were maintained on suitable medium at 4°C and subculture on Mueller Hinton Broth at 37 °C for 18 h before testing. Antibacterial activity was measured using a well diffusion method according to (Prabhu *et al.*, 2010).

#### Medium used for bacterial maintenance.

Nutrient agar medium was used to maintain the tested organisms. Bacteria were inoculated on solid medium for 24 hours at 37 °C using slope culture. Cultures were kept in refrigerator at 4 °C and they were sub-cultured every month on the same medium.

### Biological analysis

#### *Experimental animal*

The experiment was conducted on 45 male western albino rats weighting 150-180 gm were obtained from Animal House Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

The animals were housed in stainless-steel cages under strict hygienic conditions and maintained at standard laboratory conditions of temperature, relative humidity and light/dark cycle as well as a standard pelleted diet. The animals were acclimatized to laboratory conditions for 7 days prior to the commencement of the experiment .

#### *Experimental design*

The animals were divided into 6 groups , each group contain 6 rats. Group 1 as a control and the other groups (G2 – G6) were receiving the treatment according to the protocol of the research .The following protocol illustrate the number of the group and the treatment administered (see Figure 1).

**Group I:** Animals fed on normal pellet diet and receive ordinary water as control.

**Group II:** Animals were given 1.5 ml of 10% ethanol daily.

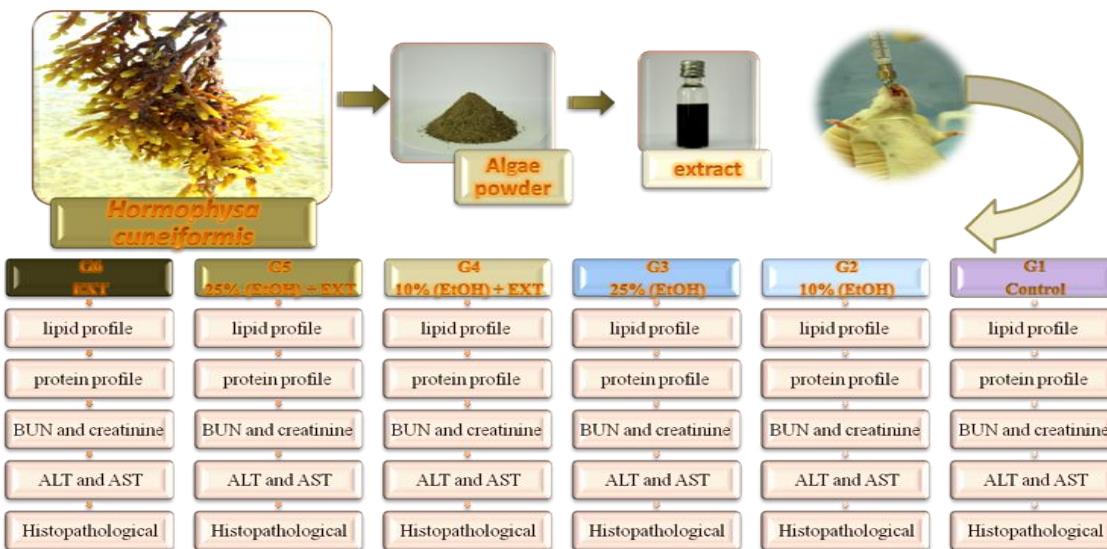
**Group III:** Animals were given 1.5 ml of 25% ethanol daily.

**Group IV:** Animals received both ethanol 1.5 ml of 10% daily and 250 mg/kg b. wt./day) of *H . cuneiformis* ethanolic extract.

**Group V:** Animals received both ethanol 1.5 ml of 25 % daily and 250 mg/kg b. wt./day) of *H . cuneiformis* ethanolic extract.

**Group VI:** Animals received 250 mg/kg b. wt./day) of *H. cuneiformis* ethanolic extract.

All the rats of various experimental groups received treatment for 30 days.The treatment was given once daily by the oral route. Blood samples were collected from 3 rats in each group before the beginning, mid and end of experiment for detection of biochemical changes.



**Fig. 1:** The protocol diagram illustrates the work plan of the biological study using the green alga *H. cuneiformis*.

## Biochemical analyses

### Blood Lipid Profiles

Total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein (LDL-C) cholesterol, and induced hepatic damage by measuring levels of Blood Urea Nitrogen BUN, Creatinine, Total proteins, albumin and Globulin and diagnostic marker enzymes such as Aspartate aminotransaminase (AST) and Alanine aminotransaminase (ALT). Blood samples were collected from the orbital sinus by use of glass capillary tube or from the tail vein by use of catheter 24 GA1.00 IN to prevent movement that will result in laceration of a blood vessel or other organ. Homeostasis was achieved by using gauze and direct pressure. For different hematological and serological studies, blood collected in a plain vacuum tube and in citrated buffered tube. Euthanasia was conducted in rats with aid of in helant anesthetic (Sevorane). The procedures of anesthesia were carried out according to method of Howard and Hughes (Howard and Hughes, 1981), while those for euthanasia were carried according to method of Lucke (1979). Sample from liver were collected from the animals and preserved in 10% formalin for histopathological studies.

### Estimation of Cholesterol (ENSURE Kit) (TC)

To 1 ml of enzyme reagent and 10 µl of plasma was mixed well and kept at 37°C for 5 minutes at room temperature and the absorbance was measured by using spectrophotometer at 505nm. The activity was calculated by using the formula:

Cholesterol conc. mg/dl = Absorbance of Test/ Absorbance of Standard x Conc. of Std (200).

### Estimation of Triglycerides (TG)

Ten µl of plasma sample mixed with 1000 µl of enzyme reagent and incubated for 5 minutes at 37°C. At the same time blank and standard solution was prepared. The absorbance of sample against reagent blank was read at 540 nm. The activity was calculated by using the formula:

Triglycerides conc. (mg/dl) = absorbance of sample/absorbance of standard × 200

### HDL-Cholesterol

To 200 µl of plasma sample 300 µl of HDL ppt reagent was added and mixed well and kept at room temperature for 10 minutes then centrifuged at 3000 rpm for 10 minutes. Then the pellet was discarded and 1ml of enzyme reagent was added to 100 µl of supernatant, then incubated for 5 minutes at 37°C and the absorbance was read at 505 nm. The activity was calculated by using the formula:

HDL Cholesterol conc. mg/dl: Abs of Test/Abs of standard x Conc of Std (50).

### Atherogenic Index (AI)

It was calculated by using the following formula:

Atherogenic index = Total cholesterol / HDL-cholesterol

### LDL-Cholesterol

The reason for choosing LDL cholesterol as a target for lipid profile is that it represents the fraction of cholesterol, which is most deleterious and has been mostly directly correlated with clinical studies. The LDL was calculated by using the formula:

LDL = (Total cholesterol) - (HDL Cholesterol) - (Triglyceride/5)

LDL cholesterol levels were expressed as mg/dl plasma.

### Risk factor

It was determined by using the formula:

Risk factor = LDL / HDL

Plasma Enzymes Profile (AST and ALT)

### Assay of aspartate transaminase (AGAPPE Diagnostics Kit)

To 1000 µl of working reagent and 100 µl of sample was added, mixed and incubate for 1 minute at 37°C and the absorbance was measured. The activity was calculated by using the formula:

ALT activity in (U/L) = ( $\Delta$  OD/min) x 1745

### **Assay of alanine transaminase (AGAPPE Diagnostics Kit)**

To 1000 µl of working reagent and 100 µl of plasma was added, mixed and incubate for 1 minute at 37°C and the absorbance was measured at 340 nm. By using the following formula the AST activity was measured.

$$\text{AST activity (U/L)} = (\Delta \text{OD/min}) \times 1745$$

### **ALT: AST Ratio.**

It was determined using the following formula.

$$\text{ALT: AST Ratio} = \text{ALT/AST}$$

### **Estimation of plasma and organs protein profile**

#### **Total protein (Autozyme kit)**

To 0.01 ml of plasma/tissues extract and 1 ml of working solution was added and incubated the assay mixture for 5 minutes at 37°C. After completion of incubation period the absorbance was measured at 546 nm. The activity was calculated by using the formula:

$$\text{Total protein in gm\%} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 5$$

#### **Total albumin (Autozyme Kit)**

To 0.01 ml of plasma and 1 ml of working solution was added and incubated the assay mixture for 1 minute at 37°C. After completion of incubation period the absorbance was measured at 600 nm. The activity was calculated by using the formula:

$$\text{Total albumin in gm\%} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 5.$$

#### **Estimation of globulin (Autozyme Kit)**

To 0.01 ml of plasma and 1 ml of working solution was added and incubated the assay mixture for 1 minute at 37°C. After completion of incubation period the absorbance was measured at 600 nm. The activity was calculated by using the formula:

$$\text{Total albumin in gm\%} = \frac{\text{absorbance of sample}}{\text{absorbance of standard}} \times 3.$$

### **Histopathological studies**

The histopathological studies were carried out to support the above parameters. At the end of experiment 2 rats from each group were sacrificed and specimens from liver were collected for histopathological studies were recorded.

After the animals were sacrificed, small parts of the liver were fixed in 10% formalin, washed in tap water, dehydrated, cleared and embedded in paraffin. Sections were cut and stained with hematoxylin and eosin for assessment the cases. To be examined under light microscopy (see Fig. 2).

### **Statistical analysis**

All results are presented as the mean ± SD. Statistical analysis was carried out by using one-way ANOVA followed by Dunnett's multiple comparison tests using Graph pad PRISM

software version. Statistical significance was considered at  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ .

### **Result and discussion**

#### **Antibacterial screening of the studied algae**

Extracts of the brown alga *H. cuneiformis* was tested against some pathogenic bacteria. The results of primary screening test are summarized in Table 1. In this study, six pathogenic bacteria were considered, namely, *S. aureus*, *MRSA*, *E. faecalis*, *E. coli*, *K. pneumonia* and *P. aeruginosa*. The crude extract of the studied marine alga exhibited different antibacterial activities and inhibited all tested bacteria. The maximum biological activities were observed against *P. aeruginosa*. Where the inhibition zone reached 40 mm. Alga extract also responsible for the observed effects against the other pathogenic bacteria.

These results indicate that the extracts contained different antibacterial substances and reflect the variety of secondary metabolites (Patterson et al., 1994). The compounds responsible for the antimicrobial activity assume that the active compounds could be at least partly, lipophilic halogenated compounds. Halogen-containing terpenoids, acetylenes and phenols have been identified in several algal species as biologically active compounds (Vairappan et al., 2001). Besides halogenated compounds, fatty acids have been identified as antimicrobial substances in algae (Rosell and Srivastava 1987). Many compounds of marine algae show anti-bacterial activities such as polysaccharide (Laurienzo 2010), lyngaroside(Ali et al., 2002), polyhydroxy lated fucophlorethol (Sandsdalen et al., 2003), bromophenols (Oh et al., 2008), guianenesquiterpene (Chakraborty and Lipton 2010), lactone malyngolide (Cardelina et al., 1979) cycloeudesmol (Sims et al., 1975), polyphenolic compound (Devi 2008), halogenated compound (Vairappan, 2003) and quinone metabolite (Horie et al., 2008).

Antibacterial activities of compounds derived from algae have also been extensively studied by several other researchers (Dmirel et al., 2009, Rajasulochana et al., 2009, Seenivasan et al., 2010).

### **Pharmacological Studies**

#### **Effect of *H. cuneiformis* extract on the lipid profile of ethanol-administrated rats,**

#### **Effect of *H. cuneiformis* extract on the TC**

When reviewing the results of the impact of injection with the brown alga *H. cuneiformis* extract on administrated rats (Table 2), we conclude that, the injecting alcohol (10 and 25 %) causes a significant increase in the TC levels for rats, while the injection with the brown alga *H. cuneiformis* extract significantly reduce this levels which appeared anti-hyperlipidemic properties. The results also showed that, the second group which treated with 10 % ethanol showed a significant increase in the TC level ( $P < 0.01$ ) reached 18.59 and 30.36% after 15, 30 days, respectively. Also, the 3<sup>rd</sup> group which treated with 25%ethanol showed significant increasing in the level of TC ( $P < 0.01$ ) reached

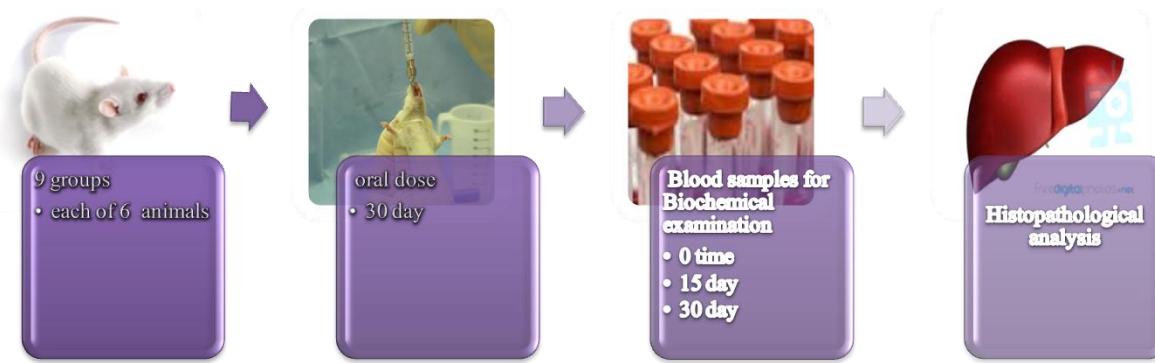
25.54 and 34.60 % after 15, 30 days of injection, respectively. On the other hand, the fourth and fifth groups which treated by 10 or 25 % ethanol together with the *H. cuneiformis* extract showed insignificant increases in TC levels reached 5.23, 3.08% for the fourth member after 15 and 30 days of injection, respectively and 1.37% for members of fifth group after 15 days of injection. On the contrary, there is a decreasing in the TC levels for the members of this group after 30 days of injection reached 2.27%. It is also surprising that the injection by *H. cuneiformis* extract of those groups which previously injected by alcohol has led to a decrease in the rate of TG levels reached 13.36, 27.28% in the fourth group and 24.17, 36.87% in the fifth group after 15 and 30 days, respectively. Additionally, the injection with *H. cuneiformis* extract for the members of the sixth group will effective in reducing the rate of TC, where TC level decreased by 4.71, 9.71% after 15, 30 days of the injection, respectively.

#### *Effect of H. cuneiformis extract on the TG*

Regarding results contained in the Table.2, and comparing the results of the treated groups (2-6) with the control group, we found different behaviors in triglyceride levels. Where the members of the second group reflect significant ( $P<0.05$ ) increasing in TG levels reached 8.91% after 15 days and recorded a significant ( $P<0.01$ ) increasing reached 59.07% after 30 days of injections. While the treatment of the third group with 25% ethanol leads to the significant (at  $P<0.01$ ) increasing in TG levels reached 21.72 and 70.79% after 15, 30 days of treatment, respectively. On the other hand, the fourth and fifth groups exhibit insignificant increasing in TG levels reached 6.71 and 8.83% for members of the fourth group and 3.93, 7.31% for the member of the fifth group after 15, 30 days of treatment, respectively. Comparing the increase in the levels of triglycerides in the rats of treated groups (2-5), we found the treatment with *H. cuneiformis* extract addition to being injected previously with alcohol has led to a decrease in the alcohol action (elevation of TG) by 2.74 and 50.24% for members of fourth group and 17.79, 63.48% for the member of the fifth group after 15, 30 days of injection, respectively. Finally, the results of the sixth group treated with *H. cuneiformis* extract showed insignificant decrease in the level of triglycerides reached 2.17 and 2.52% after 15 and 30 days of the injection, respectively. Most cholesterol in the body is present as essential components of the cell membrane, while the remainder is in transit through the blood or functions as a starting material for the biosynthesis of bile acid, steroid hormones, and vitamin D (170). Increased cholesterol levels in the serum facilitate the development of coronary atherosclerosis (Berliner and Heinecks 1996). In addition, TG is a major component of chylomicron and very low-density lipoprotein (VLDL), both of which are energy substrates for liver and peripheral tissue, particularly, muscles. High levels of TG are a risk factor for atherosclerosis (John *et al.*, 2005). In the present study, the metabolic and pharmacological effects of *H. cuneiformis* was investigated. Blood lipids, cholesterol and triglycerides were shown to be decreased after the administration of the alga extract. This is an important finding

since decreased levels of cholesterol and total lipids minimize the incidence of many cardiovascular problems (Uma *et al.*, 2011). The use of marine alga as a diet may be responsible for the relatively normal ranges of lipid profile in this region (Al-Harthi and El-Deek, 2012). Literature survey indicates that alga contain unsaturated fatty acids more than saturated fatty acid, the main fatty acid being cholesterol and many other sterols (Aliya *et al.*, 1991). Ingestion of diets containing highly unsaturated fatty acids has been shown to depress blood cholesterol level (Li *et al.*, 2008). Also, Ahmed *et al.* (1993) reported the antihypertensive effects of seaweeds contain unsaturated fatty acids more than saturated fatty acid, the main fatty acid being cholesterol and many other sterols. Ingestion of diets containing highly unsaturated fatty acids has been shown to depress blood cholesterol level (Li *et al.*, 2008).

The results indicated that the decrease in the concentration of triglycerol in the serum by dietary of the two algae was due to the promotion of fatty acid oxidation in rat liver. Since the increase in the concentration of triglycerol in the serum leads to the increase of the incidence of arteriosclerosis. Algae may play a role in the prevention of arteriosclerosis. It is harmony with finding of Murata and Nakazoe (2001), who report the dietary of Wakame is associated with the promotionoxidation of fatty acids in the liver and serum and subsequently play a role in the prevention and treatment of arteriosclerosis. An abundance of evidence strongly suggests that increased intakes of long chain omega-3 fatty acids can markedly reduce the risk of heart disease. The omega-3 fatty acids (indicates that the first double bond is located at the third carbon from the end of the fatty acid chain) such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and alpha-linolenic acid (ALA), believed to be largely responsible for their effects. These "good fats" are naturally present in some marine algae. Omega-3 fatty acids may help prevent heart disease through a number of different mechanisms. Omega-3 fatty acids also reduce the tendency to thrombosis (formation of blood clots), and thus help prevent myocardial infarction. EPA and DHA also have several actions that inhibit the development of atherosclerosis. While these fatty acids do not lower plasma cholesterol levels, they do have a substantial triglyceride-lowering effect and also raise levels of HDL "good cholesterol" (Connor2001). FT-IR spectrum of powder and ethanolic extract of powder and ethanolic extract of *H. cuneiformis* showed indicate the presence of aromatic and aliphatic amines, hydroxyl sulfonate, amino, and chloroalkanes. Furthermore, the phyco-chemical analysis of this alga reported the presence of proteins, vitamins, flavonoids, terpenoids, carotenoids, lignin, sterols, peptides and polysaccharides. Many of these compounds such as flavonoids, polysaccharides (fucodian), vitamins and terpenoids are powerful antioxidants. It was known that marine algae contain numerous bioactive substances that have been shown to lower cholesterol, reduce blood pressure, and promote healthy digestion and antioxidant activity (Fung *et al.*, 2013). The sulphur compounds in the extracts are capable of reducing the excessive accumulation of intracellular triglycerides (Dianzani, 1978).

**Fig. 2:** The protocol diagram illustrates the collection of blood samples and histopathology.**Table 2:** Effect of *H. cuneiformis* ethanolic extract on Cholesterol (TC) and Triglycerides (TG) (mg/dl) of ethanol-administered rats.

Groups Treatments	Cholesterol (mg/dl)			Triglycerides (mg/dl)		
	Zero Time	15 day	30 day	Zero Time	15 day	30 day
<b>Control</b>	58.02 ± 2.61	59.31 ± 0.55	61.25 ± 1.45	36.64 ± 1.66	36.70 ± 1.42	36.34 ± 3.20
<b>10% Ethanol</b>	60.31 ± 2.33	71.52 ± 2.01 ** (18.59%↑)	78.62 ± 3.45 ** (30.36%↑)	59.50 ± 1.00	64.80 ± 5.50 * (8.91%↑)	70.55 ± 4.23 ** (59.07%↑)
<b>25% Ethanol</b>	60.41 ± 1.74	75.84 ± 3.62 ** (25.54%↑)	81.31 ± 5.45 ** (34.60%↑)	64.08 ± 2.11	78.00 ± 5.24 ** (21.72%↑)	86.43 ± 5.23 ** (70.79%↑)
<b>10% Ethanol + Alga extract</b>	61.04 ± 2.11	64.23 ± 2.14 (5.23%↑)	62.92 ± 4.32 (3.08%↑)	49.40 ± 1.30	52.45 ± 4.00 (6.17%↑)	53.76 ± 3.21 (8.83%↑)
<b>25% Ethanol + Alga extract</b>	54.75 ± 1.64	55.51 ± 1.41 (1.37%↑)	53.51 ± 1.41 (2.27%↓)	52.70 ± 2.40	54.77 ± 1.45 (3.93%↑)	56.55 ± 3.01 (7.31%↑)
<b>Alga extract</b>	59.85 ± 1.44	57.03 ± 2.85 (4.71%↓)	54.04 ± 2.14 *	53.50 ± 2.01 (9.71%↓)	54.66 ± 2.84 (2.17%↑)	54.85 ± 3.54 (2.52%↑)

All values are mean ± SD, n=6, \* P< 0.05, \*\* P< 0.01 when compared by the values at zero time (one way ANOVA followed by Dunnett's test); Number in the parenthesis indicates % decrease or increase in the respective serum levels. ↑ denotes increase, ↓denotes decrease.

**Table 3** Effect of *H. cuneiformis* ethanolic extract on HDL, LDL of cholesterol induced hyperlipidemic rats.

Groups Treatments	HDL (mg/dl)			LDL (mg/dl)		
	Zero Time	15 day	30 day	Zero Time	15 day	30 day
<b>Control</b>	27.23 ± 0.56	28.44 ± 1.02	28.09 ± 1.02	39.44 ± 1.24	39.50 ± 1.05	39.41 ± 0.89
<b>10% Ethanol</b>	34.25 ± 0.66	27.20 ± 0.56 * (20.58%↓)	20.00 ± 0.64 ** (41.61%↓)	34.00 ± 0.55	40.65 ± 1.80 * (19.56%↑)	54.51 ± 1.05 ** (60.32%↑)
<b>25% Ethanol</b>	41.00 ± 0.78	24.00 ± 1.00 ** (41.46%↓)	22.64 ± 0.54 ** (44.78%↓)	37.54 ± 1.08	54.84 ± 1.35 ** (46.08%↑)	66.46 ± 2.34 ** (77.04%↑)
<b>10% Ethanol + Alga extract</b>	42.50 ± 0.85	39.55 ± 0.54 (6.94%↓)	41.02 ± 1.22 (3.48%↓)	52.52 ± 1.32	53.00 ± 2.50 (0.91%↑)	52.00 ± 3.40 (0.99%↓)
<b>25% Ethanol + Alga extract</b>	38.05 ± 0.49	35.50 ± 1.03 (6.70%↓)	34.02 ± 1.34 (10.59%↓)	46.54 ± 1.07	47.02 ± 1.02 (1.03%↑)	48.45 ± 2.05 (4.10%↑)
<b>Alga extract</b>	45.00 ± 0.62	55.00 ± 2.00 ** (22.22%↑)	50.00 ± 2.54 * (11.11%↑)	55.00 ± 2.74	50.50 ± 2.03 * (8.18%↓)	50.00 ± 2.00 * (9.09%↓)

All values are mean ± SD, n=6, \* P< 0.05, \*\* P< 0.01 when compared by the values at zero time (one way ANOVA followed by Dunnett's test); Number in the parenthesis indicates % decrease or increase in the respective serum levels. ↑ denotes increase, ↓denotes decrease.

#### Effect of *H. cuneiformis* extract on the HDL

Concerning the comparison between the data recorded in Table 3, we found that, HDL levels were decreased in groups (2-5) compared with control group (1<sup>st</sup> group). Whereas, HDL levels were significantly (P<0.05) decreased to 20.58 % and highly significantly (P<0.01) decreased to 41.61 % in the 2<sup>nd</sup> group by the day 15 and 30, respectively. Furthermore, HDL levels in the rats of the 3<sup>rd</sup> group were significantly (P<0.01) decreased by 41.46 and 44.78 % at the end of 15 and 30 day, respectively. On the other hand, the members of the fourth and fifth groups were exhibit insignificant decreasing in the DHL levels by 6.94, 3.48 % in the fourth group and by 6.7, and 10.59 % in the fifth group at the end of days 15 and 30, respectively. Also, the treatment of the rats

(which previously injected by 10 or 25 %) by *H. cuneiformis* extract in the fourth and fifth groups caused highly suppression in the ethanol action by 13.64 and 38.13 % in the fourth group and by 34.76 and 34.19 % in the fifth group at the end of 15 and 30 day, respectively. In contrast, the sixth group revealed a significant increasing in DHL levels at P<0.01 reached 22.22 % in the day 15 and 11.11 % at P<0.05 in the end of day 30.

#### Effect of *H. cuneiformis* extract on the LDL

Concerning the LDL levels in the experimental rats in the six groups (Table 3), we found that the results of groups (2-6) exhibit a significant increasing in the proportion of LDL level in most treatments. It reached significant increasing reached 19.56 %

at  $P < 0.05$  after 15 days of injections for the second group which reached 60.32% at  $P < 0.01$  after 30 days of injections, while these levels increased significantly ( $P < 0.01$ ) with the members of the third group reached 46.08 and 77.04% after 15, 30 days of injection, respectively. Excludes from this elevation in the LDL level that happened within members of the sixth group who's injected by *H. cuneiformis* extract. This group exhibit significant decreasing in their LDL levels ( $P < 0.05$ ) arrived 8.18, 9.08% after 15, 30 days, respectively. On the other hand, the injection of the fourth and fifth groups with extract of *H. cuneiformis* (which has already injected with ethanol by 10 or 25%) decrease the negative impact of ethanol on LDL levels reached 18.65, 59.33 % and 45.05, 72.94% in the fourth and fifth group after 15, 30 days of injection, respectively.

#### **Effect of *H. cunriformis* ethanolic extract on various biological parameters of cholesterol induced hyperlipidemic rats.**

Regarding the biological indexes for the cholesterol induced hyperlipidemic rats, we found that the groups (4-6) treated with the extract of *H. cuneiformis* demonstrated significant decrease in the Atherogenic index and LDL/HDL risk factor when compared to cholesterol induced hyperlipidemic groups (2,3) (see Table 4). The extracts of the two studied algae induced an increase in serum HDL levels in the hyperlipidemic models. During blood circulation, HDL mediates the transfer excess cholesterol from the peripheral cells to the liver for its catabolism by a pathway termed as "reverse cholesterol transport" hence increased serum HDL levels may prove beneficial in lipid disorders and might also serve as a cardio-protective factor to prevent the gradual initiation of atherosclerotic process.

**Table. 4:** Effect of *H. cuneiformis* ethanolic extract on Atherogenic Index (AI) and Risk Factor after 30 days of treatment in ethanol-administered rats.

Groups Treatments	Atherogenic Index TC / HDL	Risk Factor LDL / HDL
Control	2.18	1.4
10% Ethanol	3.93	2.73
25% Ethanol	3.59	2.94
10% Ethanol + Alga extract	1.53	1.27
25% Ethanol + Alga extract	1.57	1.42
Alga extract	1.08	1.00

According to many studies, LDL-C is the most important factor among the serum lipids. For example, LDL-C is known to accumulate in the extracellular sub-endothelial space of arteries and to be toxic to arterial walls (Rota *et al.*, 1998). Furthermore, elevated LDL-C levels play a crucial role in the development of atherosclerosis lesions that progress from fatty steaks to ulcerated plaques. Therefore, the serum level of LDL-C is used as the basis for initiating and monitoring the treatment of patients with elevated blood cholesterol levels (Schaefer *et al.*, 1995). Thus, lowering the abnormally increased serum triglyceride, as well as the TC and LDLC levels, reduces the incidence of atherosclerosis. The AI which is the ratio of LDL-C to HDL-C, is commonly used as an index to evaluate the risk for atherosclerosis as a result of increased HDL-C levels (Fki *et al.*, 2005). Therefore, elevating the

level of HDL-C and lowering the AI are important measures in reducing the risk of atherosclerosis. HDL-C exerts an anti-atherogenic effect by counteracting LDL-C oxidation and facilitating the translocation of cholesterol from peripheral tissue such as arterial walls to the liver for catabolism (Choi *et al.*, 1991). The induction of hyperlipidemia by a high-cholesterol diet in experimental animals has long been used to assess the beneficial effects of hyperlipidemic agents on the regulation of cholesterol (Yokozawa *et al.*, 2006).

#### **Effect of *H. cuneiformis* ethanolic extract on the levels of ALT**

Serum marker enzyme in ethanol-treated rats groups (G 2 and 3) compared to control group (G1) showed significant ( $P < 0.01$ ) increase in serum ALT by 32.39, 48.19 % and 47.42, 42.51 % for G2 and G 3 on day 15 and 30, respectively (Table 5). The activities of ALT rats fed simultaneously with ethanol and *H. cuneiformis* ethanolic extract (G4 and G5) exhibit insignificant increase in the serum ALT by 4.2 and 7.52 % in the G4 after 15 and 30 days of treatment, respectively. Meanwhile in the case of G5, the level of serum ALT showed reduction by 2.47 % after 15 days followed by an elevation to 2.13 % on day 30. On the other hand, the activity of ALT in the rats fed only with *H. cuneiformis* extract showed marked reduction by 6.42 % after 15 days followed by slightly increasing by 0.22 % on the day 30. Furthermore, the treatment of the G4 and G5 by the *H. cuneiformis* extract showed marked reduction in the ethanol action by 28.19, 40.67 % in G4 and by 49.89, 40.38% in G5 after 15 and 30 days, respectively.

#### **Effect of *H. cuneiformis* ethanolic extract on the levels of AST**

Regarding the AST levels of the treated groups as indicated in Table 5, we found that groups 2 and 3 exhibit significant ( $P < 0.01$ ) increasing reached 30.70, 72.80 % and 34.41, 65.35 % after 15 and 30 days of injection, respectively. While, the fourth group exhibit significant ( $P < 0.05$ ) increase in AST levels reached 10.22% and insignificant increase reached 2.59 after 15, 30 days, respectively. By the same manner, the fifth group exhibit insignificant increase reached 8.13 % after the day 15, but appeared insignificant decreasing in the AST level reached 1.97% after the day 30. On the other hand, the sixth group revealed insignificant decreasing in the AST level reached 4.39 and 6.55% after 15 and 30 days of treatment, respectively. Furthermore, the treatment of the G4 and G5 by the *H. cuneiformis* extract showed marked reduction in the ethanol action by 20.48, 70.21 % in G4 and by 26.28, 67.28 % in G5 after 15 and 30 days, respectively.

Regarding the ratio of serum alanine aminotransferase (ALT) to serum aspartate aminotransferase (AST), it was found that the ethanol-treated rats exhibit a decreasing values (ALT/AST  $< 1$ ), severe hepatic or alcoholic hepatic disease. On the other hand, this ratio exhibit normal values with rats fed simultaneously with ethanol and *H. cuneiformis* extract ( $2 < \text{ALT/AST} > 1$ ) which referred to normal individuals. The liver enzymes are normally found in circulation in small amounts because of hepatic growth and repair. ALT and AST activities were elevated in hypercholesterolemic groups, meanwhile, the treated groups

**Table 5:** Effect of ethanol extract of *H. cuneiformis* ethanolic extracts on serum hepatic markers of ALT and AST (U/L) ethanol-administered rats and ALT/AST ratio.

Groups Treatments	ALT (U/L)			AST (U/L)			ALT/AST Ratio
	Zero Time	15 day	30 day	Zero Time	15 day	30 day	
Control	59.64 ± 1.42	58.13 ± 1.20	57.68 ± 1.52	54.31 ± 0.35	54.35 ± 1.05	56.35 ± 1.09	1.33
10% Ethanol	58.35 ± 0.58	77.25 ± 1.25 ** (32.39%↑) (48.19%↓)	86.45 ± 2.81 ** (42.51%↑) (47.42%↑)	52.32 ± 0.54	68.38 ± 2.02 ** (30.70%↑) (72.80%↑)	90.41 ± 1.04 ** (65.35%↑) (2.59%↑)	0.96
25% Ethanol	63.45 ± 2.34	93.54 ± 3.24 ** (47.42%↑) (42.51%↑)	90.42 ± 2.64 ** (2.13%↑) (8.13%↑)	58.33 ± 0.76	78.40 ± 2.03 ** (34.41%↑) (10.22%↑)	96.45 ± 1.06 ** (1.97%↓) (6.55%↓)	0.94
10% Ethanol + Alga extract	48.52 ± 0.55	50.56 ± 1.65 (4.20%↑) (7.52%↑)	52.17 ± 0.68 (2.47%↓) (2.13%↑)	39.32 ± 0.05	43.34 ± 1.04 * (12.59%↑) (4.39%↓)	40.34 ± 1.04 (2.59%↑) (6.55%↓)	1.49
25% Ethanol + Alga extract	53.44 ± 1.40	52.12 ± 0.85 (2.47%↓) (2.13%↑)	55.31 ± 1.52 (2.13%↑)	49.34 ± 0.08	53.35 ± 2.06 (8.13%↑) (1.97%↓)	48.37 ± 1.08 (1.97%↓)	1.14
Alga extract	63.20 ± 1.07	59.14 ± 0.15 (6.42%↓) (0.22%↑)	63.34 ± 1.50 (0.22%↑)	45.35 ± 0.04	43.36 ± 1.03 (4.39%↓)	42.38 ± 0.85 (6.55%↓)	1.50

All values are mean ± SD, n=6, \* P<0.05, \*\* P<0.01 when compared by the values at zero time (one way ANOVA followed by Dunnett's test); Number in the parenthesis indicates % decrease or increase in the respective serum levels. ↑ denotes increase, ↓ denotes decrease.

showed an obvious enhancement (Sudhahar *et al.*, 2007). The increasing in the levels of diagnostic marker enzymes (ALT and AST) in plasma of ethanol-feeding rats as compared to that of Group I (control rats) is indicative of the cellular leakages and loss of functional integrity of cell membrane in liver. The present observation is in agreement with earlier reported studies (Kume *et al.*, 2006), which have shown that the amount of diagnostic marker enzymes present in plasma is directly proportional to the number of necrotic cells present in the liver tissue.

Oral pre-treatment with the two algal extracts attenuated the EtOH-induced elevation in the levels of these diagnostic marker enzymes in plasma of Group II and III animals as compared to other groups of rats, demonstrating the cytoprotective activity of the two algae. These findings indicated that the ethanol extract of the two algae preserved the structural integrity of the hepatocellular membrane and liver cell architecture damage caused by EtOH. Previous studies have shown that natural antioxidant molecules impart stabilization to cell membranes in relation to the degree of their free radical scavenging ability (Ganesan *et al.*, 2007).

Hence, it is possible that likewise the two algae may also prolong the viability of liver cell membranes from EtOH -induced necrotic damage by its membrane stabilizing action.

#### Effect of *H. cuneiformis* extract on the serum protein-profile of ethanol-administrated rats

##### Effect of *H. cuneiformis* extract on the protein contents.

There was a significant (P <0.001) decrease in the rate of protein contents (Table 6) for the second group reached 94.68% after 15 days and (P<0.05) reached 47.74% after 30 days. Also the 3<sup>rd</sup> group showed a significant (P<0.01) reduction in protein content reached 52.63, 58.90% after 15, 30 days of treatment.

The results also showed that the rate of protein decreased significantly (P<0.05) in the 4<sup>th</sup> group to 23.59% after 15 days, so that level was decline to reach 12.58% only after 30 days of treatment. Furthermore, Group 5 showed a decrease in the rate of protein degree to 13.64, 3.41% after 15, 30 days, respectively. On contrary, sixth group showed significant (P <0.01) increase in the

rate of protein reached 51.09% after 15 days and significant (P <0.001) increase reached 126.73 % after 30 days of treatment.

**Table 6:** Effect of ethanol extract of *H. cuneiformis* ethanolic extract on serum protein profile (mg/dl) of ethanol-administered rats.

Groups Treatments	Total proteins (mg/dl)		
	Zero Time	15 day	30 day
Control	8.53 ± 0.04	8.20 ± 0.01	8.23 ± 0.05
10% Ethanol	8.86 ± 0.08	5.30 ± 0.03 *** (94.68%↓) (47.74%↓)	4.63 ± 0.03 *
25% Ethanol	8.93 ± 0.03	4.23 ± 0.07 ** (52.63%↓) (58.90%↓)	3.67 ± 0.09 **
10% Ethanol + Alga extract	8.90 ± 0.03	6.80 ± 0.01 * (23.59%↓) (12.58%↓)	7.78 ± 0.04
25% Ethanol + Alga extract	8.80 ± 0.05	7.60 ± 0.05 (13.64%↓) (3.41%↓)	8.50 ± 0.08
Alga extract	5.50 ± 0.06	8.31 ± 0.06 ** (51.09%↑) (126.73%↑)	12.47 ± 0.06 ***

All values are mean ± SD, n=6, \* P<0.05, \*\* P<0.01, \*\*\* P<0.001 when compared by the values at zero time (one way ANOVA followed by Dunnett's test); Number in the parenthesis indicates % decrease or increase in the respective serum levels. ↑ denotes increase, ↓ denotes decrease.

#### Effect of *H. cuneiformis* extract on the albumin contents

The results recorded in Table 7 revealed a significant (P <0.05) decrease in the albumin contents of the second group reached 41.51, 47.17%, while the third group showed a significant (P <0.01) reduction to 55.93 and 66% after 15 and 30 days, respectively. The members of the fourth and fifth groups showed a significant (P<0.05) decrease reached 31.03, 25.86% for the fourth group and 14.82, 3.7% for the fifth group after 15, 30 days of treatment, respectively.

On the other hand Group 6 showed insignificant increase in the rate of albumin reached 15.9% after 15 days, while this increased to reach a significant (P<0.001) degree by 100% after 30 days.

#### Effect of *H. cuneiformis* extracts on the globulin contents

Regarding the globulin contents within the experimental rats (Table 7), there is a significant (P <0.05) decrease in the rate globulin in the second and third groups reached 38.2, 48.59% and 46.21, 41.91% after 15, 30 days for the two groups, respectively.

**Table 7:** Effect of *H. cuneiformis* ethanolic extract on albumin and globulin contents of ethanol-administered rats.

Groups Treatments	Albumin (mg/dl)			Globulin (mg/dl)		
	Zero Time	15 day	30 day	Zero Time	15 day	30 day
Control	5.30 ± 0.04	5.20 ± 0.04	5.30 ± 0.05	3.33 ± 0.08	3.00 ± 0.01	3.93 ± 0.02
10% Ethanol	5.30 ± 0.05	3.10 ± 0.08 *	2.80 ± 0.09 *	3.56 ± 0.04	2.20 ± 0.04 *	1.83 ± 0.01 *
		(41.51%↓)	(47.17%↓)		(38.2%↓)	(48.59%↓)
25% Ethanol	5.90 ± 0.04	2.60 ± 0.02 **	2.00 ± 0.08 **	3.03 ± 0.05	1.63 ± 0.02 *	1.76 ± 0.04 *
		(55.93%↓)	(66%↓)		(46.21%↓)	(41.91%↓)
10% Ethanol + Alga extract	5.80 ± 0.02	4.00 ± 0.01*	4.30 ± 0.02*	3.10 ± 0.03	2.80 ± 0.05	3.48 ± 0.06
		(31.03%↓)	(25.86%↓)		(9.68%↓)	(12.26%↑)
25% Ethanol + Alga extract	5.40 ± 0.04	4.60 ± 0.04	5.20 ± 0.05	3.40 ± 0.04	3.00 ± 0.04	3.30 ± 0.05
		(14.82%↓)	(3.7%↓)		(11.77%↓)	(2.94%↓)
Alga extract	3.90 ± 0.06	4.52 ± 0.06 *	7.80 ± 0.01***	2.60 ± 0.01	3.79 ± 0.07 *	4.67 ± 0.03 **
		(15.90%↑)	(100%↑)		(45.77%↑)	(79.62%↑)

All values are mean ± SD, n=6, \* P< 0.05, \*\* P< 0.01, \*\*\* P< 0.001 when compared by the values at zero time (one way ANOVA followed by Dunnett's test); Number in the parenthesis indicates % decrease or increase in the respective serum levels. ↑ denotes increase, ↓ denotes decrease.

**Table 8:** Effect of *H. cuneiformis* ethanolic extract on the serum BUN and creatinine (mg/dl) of ethanol-administered rats.

Groups Treatments	BUN (mg/dl)			Creatinine (mg/dl)		
	Zero Time	15 day	30 day	Zero Time	15 day	30 day
Control	18.50 ± 0.20	19.41 ± 0.20	20.00 ± 0.64	0.31 ± 0.05	0.35 ± 0.05	0.35 ± 0.09
10% Ethanol	20.08 ± 0.27	34.63 ± 0.14 **	29.44 ± 0.85 *	0.32 ± 0.04	0.48 ± 0.02 **	0.41 ± 0.04 *
		(73.15%↑)	(47.2%↑)		(50%↑)	(28.13%↑)
25% Ethanol	18.00 ± 0.57	33.25 ± 0.87 ***	29.50 ± 0.41 **	0.33 ± 0.06	0.48 ± 0.03 *	0.40 ± 0.06 *
		(84.72%↑)	(63.89%↑)		(45%↑)	(21.21%↑)
10% Ethanol + Alga extract	18.00 ± 0.56	21.17 ± 0.65	17.41 ± 0.12	0.32 ± 0.05	0.34 ± 0.04	0.30 ± 0.04
		(17.61%↑)	(3.28%↓)		(6.25%↑)	(6.25%↓)
25% Ethanol + Alga extract	16.40 ± 0.02	20.23 ± 0.12*	17.04 ± 0.76	0.34 ± 0.08	0.35 ± 0.06	0.30 ± 0.08
		(23.35%↑)	(3.9%↑)		(2.94%↑)	(11.77%↓)
Alga extract	18.00 ± 0.08	19.41 ± 0.85	18.50 ± 0.68	0.35 ± 0.04	0.33 ± 0.03	0.34 ± 0.05
		(7.8%↑)	(2.78%↑)		(5.71%↓)	(2.86%↓)

All values are mean ± SD, n=6, \* P< 0.05, \*\* P< 0.01, \*\*\* P< 0.001 when compared by the values at zero time (one way ANOVA followed by Dunnett's test); Number in the parenthesis indicates % decrease or increase in the respective serum levels. ↑ denotes increase, ↓ denotes decrease.

Although the fourth group showed insignificant reduction in the globulin rate (9.68%) after 15 days, but it showed insignificant increasing reached 12.26% after 30 days of treatment. On the other hand, Group 5 showed insignificant reduction in the rate of globulin to 10.77 and 2.94% after 15, 30 days of treatment, respectively. Meanwhile, the sixth group showed significant (P <0.05) increase in the globulin rate reached 45.77% after 15 days and significant (P<0.001) increase to 79.62% after 30 days of treatment. Albumin and globulin are two key components of serum proteins. Because albumin is synthesized in the liver, it is one element that is used to monitor the liver function (Friedman *et al.*, 1980).

Decreased serum total protein, albumin and globulin are seen in ethanol-administered rats of our experiment run in parallel with the finding of (Abdel-Raouf *et al.*, 2011). They demonstrated the decreased functional ability of ethanol-administered rat liver. Also, the decreased total protein concentration may be an indirect effect of the decreased albumin concentration. A significant increase in serum total protein, albumin and globulin levels was observed in algal extract co-administered rats. Stabilization of serum protein levels through the co-administration of *H. cuneiformis* extract is another additional clear indication of the improvement of the functional status of the liver cells. Damage to the liver after ethanol ingestion is a well-known phenomenon, and the obvious sign of hepatic injury is the leakage of cellular enzymes into plasma (Baldi *et al.*, 1993).

#### Effect of *H. cuneiformis* extract on the BUN and creatinine levels in blood serum

##### Effect of *H. cuneiformis* extract on the BUN

Results in Table 8 indicated a significant (P<0.01) increase in the rate of BUN in Group 2 arrived 73.15% after 15 days and significant (P<0.05) increase reached 47.2% after 30 days. While the third group exhibit a significant (P<0.001) increase reached 84.72% after 15 days followed by a significant (P<0.01) increase reached 63.89% after 30 days. At the same time, Group 4 recorded insignificant increasing in BUN by 17.61% and a contrast ratio recorded reduction in BUN to 3.28% after 30 days. On the other hand, Group 5 recorded a significant (P<0.05) increase arrived 23.35% after 15 days which declined to 3.9% after 30 days, while the sixth group recorded insignificant increasing by 7.8 and 2.78 % after 15 and 30 days, respectively. A blood urea nitrogen (BUN) test reveals important information about how well kidneys and liver are working.

A blood urea nitrogen test measures the amount of urea nitrogen that's in blood. Liver produces ammonia which contains nitrogen, after it breaks down proteins used by body cells. The nitrogen combines with other elements, such as carbon, hydrogen and oxygen, to form urea, which is a chemical waste product. The urea travels from liver to kidneys through blood stream. Healthy kidneys filter urea and other waste products from blood. The filtered waste products leave body in urine. If a blood urea

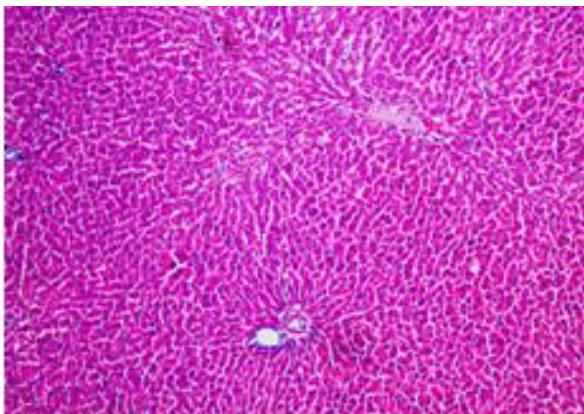
nitrogen test reveals that urea nitrogen levels are higher than normal, it probably indicates that kidneys aren't working properly. Or it could point to high protein intake, inadequate fluid intake or poor circulation. If a blood urea nitrogen test shows lower than normal levels, it could indicate liver disease or damage, or malnutrition. But a low BUN level wouldn't likely be the first indication of liver disease because the blood urea nitrogen test isn't used as a screening test for that disorder.

#### Effect of *H. cuneiformis* extract on the creatinine

Regarding the creatinine rate in the experimental rats treated or untreated with *H. cuneiformis* (Table 8), we found that, Group 2 exhibit a significantly ( $P<0.01$ ) increase reached 50% after 15 days and significant ( $P<0.05$ ) increase reached 28.13% after 30.

While the third group recorded significant ( $P<0.05$ ) increase reached 45, 21.21% after 15 and 30 days, respectively. In the same manner, Groups 4 and 5 showed insignificant increasing reached 6.25, 2.94 % after 15 days and comeback to fill down to 6.25 and 11.77 % after the day 30, respectively. On the other hand, Group 6 showed a decline percentage in the creatinine level by 5.71, 2.86 % after 15 and 30 days, respectively. Creatinine clearance calculated from creatinine concentrations in urine and plasma samples, and the urine flow rate, as well as urea clearance, is used to determine the glomerular filtration rate of the kidneys. Although not commonly done anymore, they remain useful tests for renal function.

Thus, plasma concentrations of creatinine and urea could be used as indicators of nephrotoxicity (Henry 2001). Low clearance of creatinine and/or urea indicates a diminished impaired ability of the kidneys to filter these waste products from the blood and excrete them in urine. As their clearance values decrease, their blood levels increase. Hence, an abnormally elevated blood creatinine is diagnostic of impaired renal function (Wallach 2000).

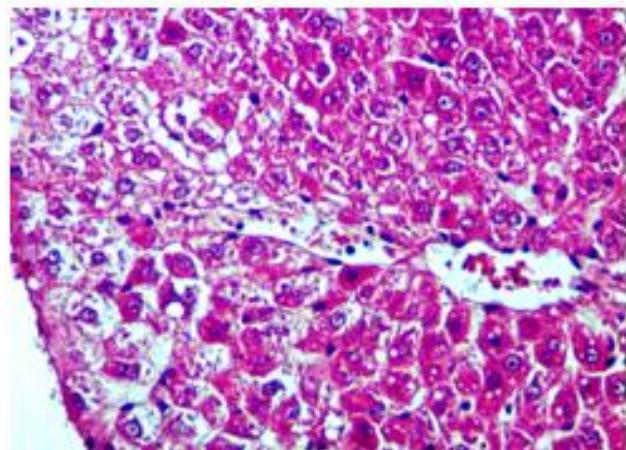


**Fig. 3:** Microscopically examination of liver of rats in negative control (Group I) showing the normal histological structures.

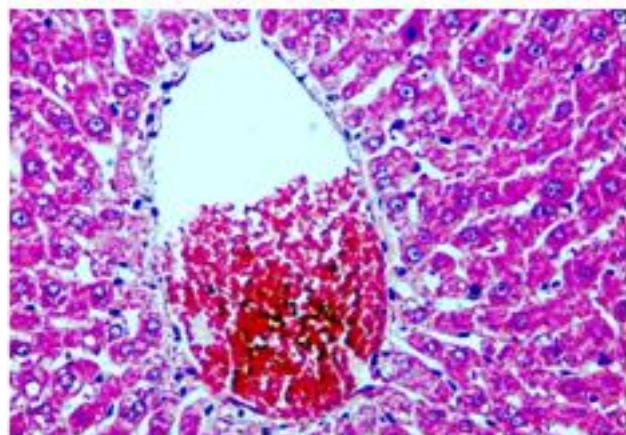
#### Histopathological studies

The histopathological examination of the ethanol treated groups of rats revealed degeneration and necrotic changes as

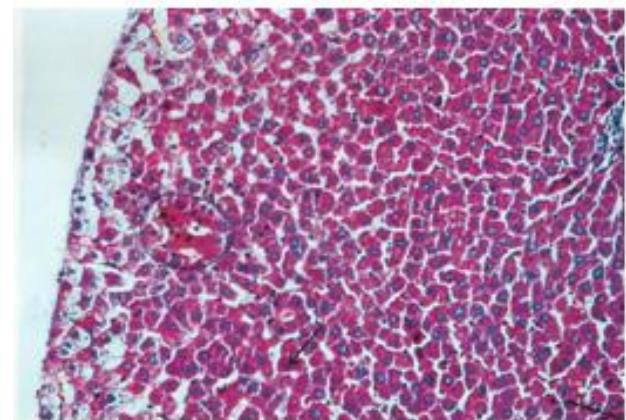
compared to the administrated rate groups with alga extract which showed marked amelioration of the severity of these changes (Figures 3-8).



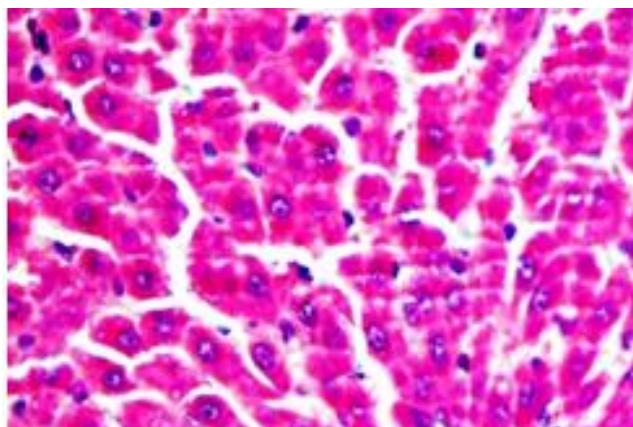
**Fig. 4:** Microscopically examination of liver of rats in Group II (treated with 10% ethanol) showing grade 2-fibrosis and definite increase with incomplete septa from the portal tract to central vein, those septa which don't interconnect with each other.



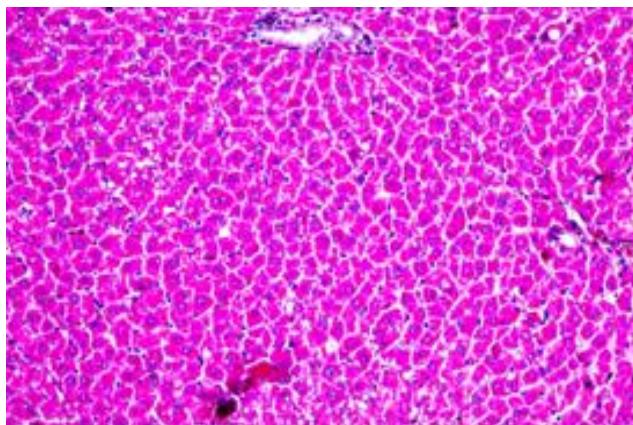
**Fig. 5:** Microscopically examination of liver of rats in Group III (treated with 25 % ethanol) showing inflammatory leukocytic cell infiltration, hydropic degeneration and haemorrhage in the central vein.



**Fig. 6:** Microscopically examination of liver of rats in Group IV (treated with 10 % ethanol + H. cuneiformis extract) showing congested blood vessels and few necroses.



**Fig. 7:** Microscopically examination of liver of rats in Group V (treated with 25 % ethanol + *H. cuneiformis* extract) showing swelling of hepatocytes and mild degeneration. In addition, focal areas of necrosis appeared.



**Fig. 8:** Microscopically examination of liver of rats in Group VI (treated with *H. cuneiformis* extract) showing normal liver without collagen or fibrosis, normal liver architecture of hepatocytes since they were well arranged without any alternation at central vein. Hepatic cells were polyhedral in shape with defined cell lining in the liver tissue. The cytoplasm was well preserved with prominent nucleus and nucleolus indicating no fibrosis.

## CONCLUSION

Brown alga *Hormophysa cuneiformis* collected from south coast of Arabian Gulf (Ad Dammam seashore), has been shown to possess a specific antimicrobial and anti-hyperlipidemic activities. These observations showed their importance as a potential source for biological active compounds. It could be concluded that extracts of *H. cuneiformis* were able to reduce all the elevated biochemical parameters and had therapeutic and preventive efficiencies in ethanol induced hepatotoxicity in rats.

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