Protective effect of *Ulva lactuca* extract during hepatic cold preservation in rats

Effet protecteur de l'extrait de l'*Ulva lactuca* sur la conservation froide du foie de rat

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RÉSUMÉ

Prérequis : Plusieurs chercheurs ont focalisé leurs travaux sur les moyens d'amélioration de la préservation des organes après ischémie froide prolongée (avant la transplantation) avec le maximum de conservation de l'activité fonctionnelle du greffon.

Objectif : Evaluer l'effet de l'extrait de l'algue verte Ulva lactuca afin d'améliorer la solution de conservation après ischémie froide hépatique chez le rat Wistar.

Méthodes : Les animaux ont été répartis en 3 groupes : un groupe Sham (courte préservation); le foie a été lavé avec une solution saline (NaCl 9 %), puis placé dans une solution de conservation (Krebs-Henseleit) pendant 30 minute à 4 °C. Pour les deux autres groupes (le groupe non-traité et le groupe traité avec l'extrait de l'Ulva lactuca), le foie a été préservé dans les mêmes conditions pendant 24 h. Après la conservation du foie, nous avons réalisé une extraction des mitochondries pour le dosage de MDA et l'évaluation de la respiration mitochondriale. Dans le modèle d'ischémie hypothermique, les activités des ASAT et des ALAT et le dosage du Na+, Ca2+, K+ et le Cl-, ont été évalué dans le liquide de conservation. Le foie a été utilisé pour l'étude histologique.

Résultats : Les taux des ASAT et des ALAT diminuent significativement chez le groupe traité (185 ± 35,2 et 163,6 ± 24,5 UI) par rapport au groupe non-traité (644,3 ± 254,4 et 925,7 ± 459,7 UI) respectivement. Le pourcentage de l'inhibition de la péroxydation lipidique a augmenté de 26,43 % ± 1,33 et le pourcentage d'activité mitochondriale a augmenté de 38,8 % ± 4,3 entre les groupes traité et non-traité. Nous avons observé une dégénérescence ballonisante diffuse (lésion réversible) et des cellules apoptotiques chez le groupe non-traité.

Conclusion : l'extrait de l'Ulva lactuca protège les hépatocytes des lésions induites par l'ischémie hypothermique chez le rat.

Mots-clés

Ischémie froide, Ulva lactuca. apoptose, nécrose, mitochondrie.

SUMMARY

Background: The researchers find means for the improvement of the organs conservation allowing prolonging the durations of the cold ischemia (before transplantation) with preservation of the maximum of their functional activity.

Aim: To evaluate the extracts stemming from the green seaweed Ulva lactuca to improve the organs conservation solution against cold hepatic-ischemia effects in Wistar rats.

Methods: Animals were randomized between 3 groups: sham group (shortly preserved); livers were washed with saline solution (NaCl 9 ‰), and then placed in a preservation solution (Krebs–Henseleit) for 30 min at 4°C. For the other two groups (non-treated group and treated group with Ulva lactuca extract), livers were preserved under the same conditions for 24 h. After liver conservation, we realized an extraction of mitochondria for the dosage of MDA and to evaluate the mitochondrial respiration. In the hypothermic ischemia model AST and ALT activities, and Na+, Ca2+, K+ and Cl- monitoring, were assessed in the preservation liquid. Fragments of livers were used for histological analysis.

Results: AST and ALT levels decrease significantly in the treated group (185 ± 35.2 and 163.6 ± 24.5 U/l) compared to the non-treated group (644.3 ± 254.4 and 925.7 ± 459.7 U/l) respectively. The percentage of lipid peroxidation inhibition increased of 26.43 ± 1.33% and the percentage of mitochondrial activity increased of 38.8 ± 4.3%, between treated and non-treated groups. Extensive ballooning degeneration (reversible lesion) and apoptosis cell was found in the non-treated group.

Conclusion: Ulva lactuca extract protects hepatocytes from the deleterious effect induced by hypothermic ischemia in rats.

Key-words

Cold ischemia; Ulva lactuca; apoptosis; necrosis; mitochondria.

In recent years, many marine resources have drew attention in the research for bio-active compounds to develop new drugs and health foods. (1) Marine algae are now considered as a rich source of antioxidants (2). It is known that seaweeds contain numerous bioactive substances that have the ability to lower cholesterol, reduce blood pressure, promote healthy digestion; and antioxidant activity (3). Natural antioxidants are interesting compounds due to their properties which help prevent oxidative stress (4), among other potentially beneficial actions. For instance, several biological effects have been attributed to flavonoids, such as anti-tumoral, anti-inflammatory, anti-ischemic and anti-aggregate plaquetary activities. These activities are believed to be in part related to the antioxidant properties of the compounds, namely in scavenging radical oxygen species (ROS). (5, 6) The cold ischemia constitute a situation of oxidative stress in touch with liberation of oxygenated radicals, these situations incited the researchers to find means for the improvement of the conservation of organs allowing to prolong the durations of the cold ischemia of certain organs (in particular the liver) with conservation of the maximum functional value. However, the constant efforts led by the teams of transplantation to develop transplants, the conservation of organs remains a problem to be resolved. (7) Conservation solution of organ appears as being a stemming to remedy the fatal effects of the ischemia-reperfusion.

For our part, we think that seaweeds have not delivered their secrets and yet especially that the marine environment of the Tunisian coast still remains little exploited in spite of the big variety of the fauna and the flora of the coast. We envisage in this work, to study a sort of seaweed collected on the Tunisian quotation in the region of «Chott Meriem» (North West of Tunisia). The purpose of our work is to estimate the capacity of extracts stemming from the green seaweed *Ulva lactuca* to improve the conservation solution of organs against the hepatic effects of ischemia.

METHODS

Plant material and extraction procedure

Ulva lactuca has been collected in February at the region of Chott Meriem. The fresh plants were sorted out and dried in the drying room with active ventilation at ambient temperature. Identification was confirmed by PR Mohamed Boussaid (Botanic Biotechnology Department of National Institute of Applied and Technology of Tunis). Plant material (*Ulva lactuca*) was reduced to a fine powder and was mixed with 40 mL of saline solution (NaCl 9 ‰). After maceration, the mixture is filtrated and centrifuged at 4000 x g for 15 min. The solid is extracted again with 5 mL of methanol during 18 hours. The methanolic extract was centrifuged at 4000 x g for 15 min. the combined filtrates are evaporated and dissolved in 5 mL of saline solution. Finally, a centrifugation at 15000 x g for 15 min was performed, and we conserved the supernatant in sterile tubes and in dark at 4°C.

Determination of total phenolic content

Total phenolic content was estimated by the Folin–Ciocalteu (FC) colorimetric method. Briefly, 50 µL of the plant extract was diluted to 10-fold with NaCl 9% and mixed with 750 µL of FC reagent, after being diluted by distilled water by 10 folds. The mixture was incubated at obscurity for 5 min at 37°C. A 750 µL of Na_2CO_3 (60 g/L) solution was added to the mixture. After 90 min, the absorbance was measured at 725 nm. A standard range was prepared in the same conditions as the samples using different concentrations of Gallic Acid.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

Due to its unpaired electron, DPPH radical gives a strong absorption band at 517 nm (deep violet color). As this electron becomes paired in the presence of a free radical scavenger, the absorption vanishes and the resulting discoloration is stoichiometric with respect to the number of electrons taken up. This test carried out by mixing 25 µL of the extract (diluted 10-fold with NaCl 9%) with 1 mL of DPPH radical to a final concentration 0.04 mM. Mixtures were vigorously shaken and let for 30 min at dark. The control corresponds to 1 mL of DPPH 0.5 mM (which was diluted in 4 ml of NaCl 9%). The antioxidant activity was expressed in percentage of the inactivated DPPH radical according to the following formula (8):

DPPH radical inactivation = 100 $(A_0 - A_s) / A_0$.

A_0: absorbance of the control at 517 nm (containing all reagents except the test compound);

A_s: absorbance of the sample at 517 nm.

Ascorbic Acid and α Tocopherol were used as reference antioxidants.

Animals

Adult male Wistar rats, weighing 250 to 350 g were provided by Tunisian Pasteur Institute and then grown in the unit of experimental Medicine of the Faculty of Medicine of Tunis. Animals were subdivided in three groups (n=5 for each one): Sham (hypothermic ischemia for 30 min), non-treated (hypothermic ischemia for 24 hours) and treated (hypothermic ischemia for 24 hours with the addition of Ulva lactuca extract in Krebs solution).

Ethic

All animal procedures used in this study are in strict accordance with the European Community Council Directive of 24 November 1986 (86-609/EEC) and Decree of 20 October 1987 (87-848/EEC).

Experimental protocol and hepatic ischemia
For hypothermic liver ischemia, rats were randomized in 3 groups. For the sham group (shortly preserved), livers were washed with 0.9% NaCl, and then placed in a preservation solution (Krebs-Henseleit) for 30 min at 4 °C. For the other two groups (non-treated group and treated group), livers were preserved under the same conditions for 24 h.For treated group, *Ulva lactuca* extract (40 µg/mL) was added to the preservation solution.
After conservation (30 min for sham group and 24h for the other groups), an extraction of mitochondria was carried out for the dosage of MDA and mitochondrial respiration study. In the hypothermic ischemia model, the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, sodium (Na^+), Calcium (Ca^{2+}), Potassium (K^+) and Chlorinates (Cl^-) levels, were assessed in the preservation liquid after conservation 30 min or 24 h. The taken liver was preserved during 12 hours in 10% of formol for histological analysis.

Isolation of mitochondria
Rat liver mitochondria were isolated as described by Lardy et al. 1967. (9) Briefly, livers were excised rapidly and placed in medium containing 250 mM sucrose, 10 mM Tris and 1 mM of the chelator EGTA, pH 7.2 at 4 °C. The tissue was scissor minced and homogenized on ice using a Teflon Potter homogenizer. The homogenate was centrifuged at 600 x g for 10 min (Sorvall RC 28 S). The supernatant was centrifuged for 5 min at 15000 x g. The mitochondrial pellet was washed with the same medium and centrifuged at 15000 x g for 5 min. The resulting mitochondrial pellet was washed with medium from which the EGTA was omitted and centrifuged for 5 min at 15000 g resulting in a final pellet containing. The protein content was determined by the method of Lowry et al. 1951 (10). The mitochondrial suspension was stored on ice before the assay of mitochondrial respiration.

Measurement of mitochondrial respiration
O_2 consumption was measured by a Clark type oxygen microelectrode (Eurosep Instruments, Cergy, France) in a thermostat controlled chamber. Mitochondria (1 mg) were added to 1.8 mL of phosphate buffer. Mitochondrial respiration was initiated by the addition of succinate (6 mM final concentration), and oxidative phosphorylation was initiated by the addition of ADP to a final concentration of 0.2 mM.
O_2 consumption recordings allowed the calculation of the substrate respiration rate (V2), the ADP-stimulated respiration rate (V3), the respiratory control ratio (RCR=V3/V2).

Malondialdehyde analysis
MDA levels in the samples were determined to obtain quantitative estimation of the membrane lipid oxidative damage. The thiobarbituric acid method was used to quantify MDA levels and estimate the lipid peroxidation in liver, measured as thiobarbituric acid-reactive substances. Briefly, mitochondrial liver (1.25 mg/mL) [11] was incubated during 40 min at 37°C. Trichloroacetic acid (3% TCA) was added to stop the peroxidation reaction, and centrifuged at 3000 x g for 15 min at 20°C. The supernatant was added to TBA 1%, followed by the incubation during 30 min at 95°C. The samples were cooled, and their absorbencies were measured by spectrophotometric method at 530 nm using NaCl 0.9% and TBA (v/v) as an external standard solution.
% lipid peroxidation = 100 x (($[MDA]_{NT}$ - $[MDA]_{T}$) / ($[MDA]_{NT}$ - $[MDA]_{Sh}$))
NT: non-treated group; **T**: treated group; **Sh**: sham.

Biochemical assay
Liver enzymes (ALT and AST) were monitored, in order to evaluate hepatic injury which was made by an automated analyzer (COBAS Integra ^{800}) using the enzymatic technique EMIT (enzyme multiplied immunoassay technique).
Results were expressed in percentage (%) of protection of AST and ALT using the following formula:
% Protection AST = 100 x ($AST_{NT} - AST_{T}$) / ($AST_{NT} - AST_{Sh}$)
The same equation was used to determine the percentage of protection of ALT.

Dosage of mineral salts
The dosage of mineral salts made with an automat the COBAS INTEGRA800®, using EMIT technique. Various measured mineral salts are: Na^+, Ca^{2+}, K^+ and Cl^-.

Histopathology
Tissues were embedded in paraffin and 4-5 mm sections were cut and stained with hematoxylin-eosin. Anatomopathologic assessment of necrosis and the apoptosis was made by photonic microscope.

Statical analysis
All values are given as means ± S.E.M. Statistical comparisons were made by using ANOVA test. A p value < 0.05 was considered statistically significant.

RESULTS

Polyphenol
The total phenols determined by the Folin-Ciocalteu method for aqueous extract of *Ulva lactuca* was 25.65 ± 2.82 mg of Gallic Acid /g dry plant material. The total phenolic compound content in plant extract is shown in table 1.

DPPH
In order to evaluate the antioxidant capacity of the samples, a method based on the reduction of DPPH was

performed. DPPH radical is one of the few stable organic nitrogen free radicals, which has been widely used to determine the free radical-scavenging ability of various samples. Results of DPPH reduction by the *Ulva lactuca* extract, Ascorbic Acid and α Tocopherol solution are shown in table 1.

Table 1 : Dosage of total phenols, percentage of DPPH inhibition and percentage of lipid peroxidation inhibition (in vitro) of Ulva lactuca extract compared to Ascorbic Acid and α Tocopherol.

	Total phenols (mg GA/g DPM)	% of DPPH inhibition	% of lipid peroxidation inhibition (in vitro)
Ulva lactuca extract	25.65 ± 2.82	11.13 ± 2.75	0
Ascorbic Acid	-	92.98 ± 1.77	68.92 ± 5.9
α Tocopherol	-	87.42 ± 4.34	60.04 ± 0.26

Values are expressed as mean ± standard deviation.

Quantitative analysis revealed that the Ascorbic Acid and α Tocopherol solutions showed the strongest DPPH radical scavenging property (92.98 ± 1.77% and 87.42 ± 4.34% respectively). *Ulva lactuca* extracts present a low antioxidant activity (11.13 ± 2.75%).

Serum transaminases levels

The ALT and AST serum levels of the sham group were 9.2 ± 4.32U/l and 0 U/l respectively. Serum ALT and AST levels increased significantly after cold ischemia for Non-treated group compared with Sham group (644.3 ± 254.4U/l and 925.7 ± 459.7 U/l respectively).

Pretreatment with *Ulva lactuca* significantly decreased AST and ALT levels, respectively, 185.3 ± 35.2 U/l and 163.6 ± 24.5 U/l, compared with Non-treated group ($p=0.03<0.05$). (Table 2)

Lipid peroxidation inhibition

In vitro

The antioxidant properties were cheeked of *Ulva lactuca*, Ascorbic Acid and α Tocopherol on isolated liver mitochondria from control animals.

Lipid peroxidation was induced by the addition of mixture of Fe^{2+}/Fe^{3+} in the absence or in the presence of Ulva extract.

Ascorbic Acid and α Tocopherol solutions inhibited lipid peroxidation (68.92 ± 5.9% and 60.04 ± 0.26% respectively), Ulva extract can't inhibited this damage. (Table 1)

Ex vivo

Interestingly, antioxidant properties were observed ex vivo in the rat liver model after 24 h cold preservation treated with *Ulva lactuca* (26.43 ± 1.33%). (Table 4)

Indeed, the MDA content as was significantly higher for Non-treated group (21.84 ± 2.08 µM) than in sham group (1.38 ± 0.36 µM).

For Treated group, MDA decreased 16.44 ± 0.27 µM compared with non-treated group. (Table 3)

Table 3 : Measurement of mitochondrial respiration and malondialdehyde analysis

	V3 (nM /mg/min)	V2 (nM/mg/min)	RCR = V3/V2	MDA (µM)
Sham	94.18 ± 3.3	36.9 ± 1.5	2.56 ± 0.13	1.38 ± 0.36
Non-treated	40.04 ± 2.9 *	38.8 ± 2	1.03 ± 0.02 *	21.83 ± 2.08 *
Treated	57.2 ± 1.53 ¥	35.3 ± 1.3	1.62 ± 0.1 ¥	16.44 ± 0.27 ¥

Values are expressed as mean ± standard deviation (n = 5) at $p < 0.05$. ADP-stimulated rate (V3), substrate stimulation rate (V2), respiratory control ratio (RCR) and Malondialdehyde (MDA).
* Significantly different from sham versus non-treated group at $p < 0.05$;
¥ Significantly different from non-treated group versus treated groups at $p < 0.05$.

Table 4 : Effect of *Ulva lactuca* extract on the biochemical parameters in cold ischemia liver (treated group versus non-treated group)

	Percentage (%)
AST reduction	72.3 ± 5.54
ALT reduction	94.92 ± 8.5
Mitochondrial protection	38.8 ± 4.3
Inhibition of lipoperoxidation	26.43 ± 1.33

Values are expressed as mean ± standard deviation (n = 5). Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST).

Dosage of mineral salts:

We evaluate the biochemical parameters in Krebs-Henseleit solution before and after preservation.

The addition of algal extract significantly decreased Potassium level compared to the non-treated group. (Table 2)

Table 2 : Biochemical parameters in Krebs–Henseleit Solution before and after preservation

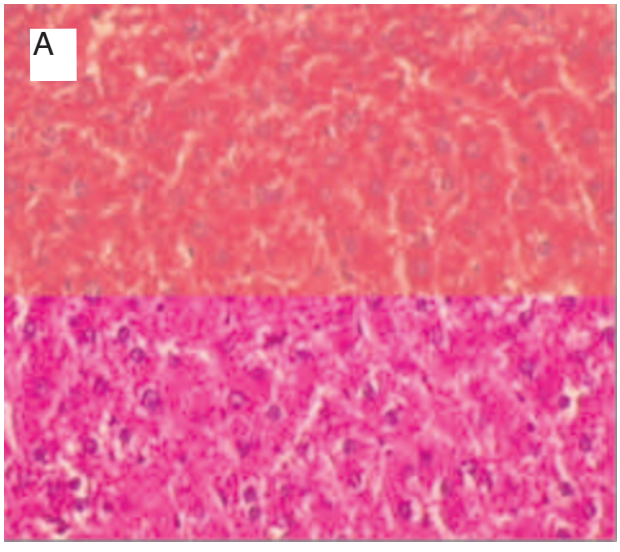
	AST (UI)	ALT (UI)	Na+ (µmol/l)	K+ (µmol/l)	Cl- (µmol/l)	Ca2+ (µmol/l)
Krebs–Henseleit Solution	0	0	142	5.5	123	1.1
Sham	9.2 ± 4.32	0	141 ± 27.9	5.6 ± 0.52	121 ± 12.7	1.36 ± 0.07
Non-treated	644.3 ± 254.4*	925.7 ± 459.7 *	137 ± 28.6	18.4 ± 1.05 *	121 ± 17.2	1.48 ± 0.25
Treated	185 ± 35.2 ¥	163.6 ± 24.5 ¥	138.3 ± 1.92	10.3 ± 1.8 ¥	124.5 ± 2.3	1.4 ± 0.04

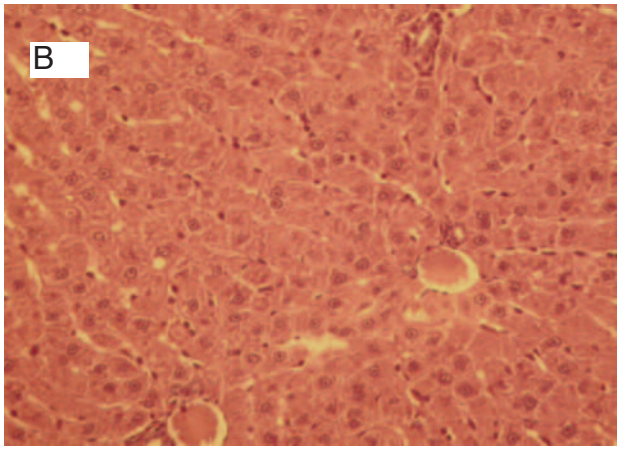
Values are expressed as mean ± standard deviation (n = 5) at $p < 0.05$. Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), sodium (Na+), Calcium (Ca2+), Potassium (K+) and Chlorinates (Cl-).
* Significantly different from sham versus non-treated group at $p < 0.05$;
¥ Significantly different from non-treated group versus treated groups at $p < 0.05$.

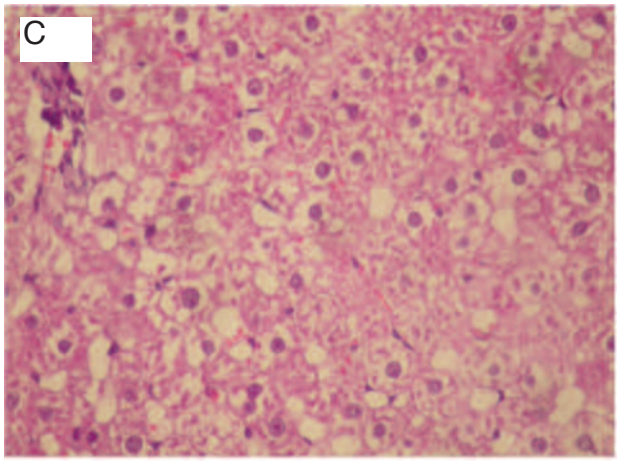
Measurement of mitochondrial respiration:
The addition of ADP to mitochondria, containing succinate as substrates, stimulates the respiration from state 3 to state 4 and when all the added ADP is phosphorylated, it shifts back to state 4 (ADP exhausted) respiration.
We studied the progress of mitochondrial parameters after 24 h in cold storage. RCR is the ratio of state 3 to state 4 respirations.
24h cold ischemia hugely altered oxidative phosphorylation parameters as demonstrated by extensive decrease in state 3 respiration rate (40.04 ± 2.9) and respiratory control ratio RCR (1.03 ± 0.02). (Table 3)
The presence of *Ulva lactuca* in the preservation solution limited the drop in respiratory control ratio by both increasing the state 3 respiration rate (57.2 ± 1.53) and slightly lowering the state 4 respiration rate(35.3 ± 1.3), the latter was not statistically significant. (Table 3)

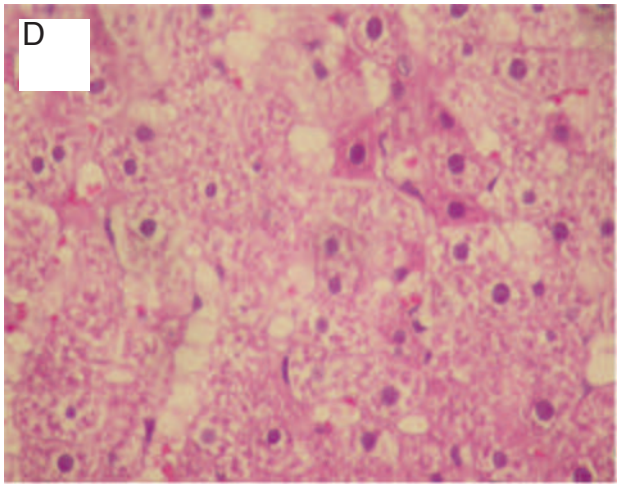
Histological Assessment
In this study, there was marked a histological difference in the damaged liver, evaluated by sector of necrosis, between sham-operated liver and liver subjected to 24 hours of cold ischemia and/or *Ulva lactuca* extract treatment.
The Sham and treated group did not show any morphologic changes (Figures 1 : A and B). Treatment with plant extract preserved the normal morphology of the liver demonstrating normal hepatocytes (Figures 1 : B).
In contrast, the section of liver obtained from non-treated group demonstrated features of severe damage.

Figure 1: liver section of sham rats showing normal hepatocytes; (B) liver section of Ulva lactuca + cold ischemia (treated group) shows normal histology; (C) and (D) liver section of non-treated group shows Extensive ballooning degeneration in the periportal, midzonal and pericentral regions and cellular apoptosis. (H and E staining, magnification, x 400)
All histologic evaluation was done in a double blinded fashion.









These features included extensive ballooning degeneration in the midzonal, periportal and pericentral regions (Figure C) and apoptotic cell (Figure D).

DISCUSSION

Cold ischemia injury is one of the major obstacles to liver transplantation. In clinical setting 15 % to 25 % of livers allograft do not show full functional recovery when reperfused, and as many as 6 % of them fail post operatively for no known reason. (12) The conservation solution plays an important role in process of cold hepatic ischemia, since its objective is to provide the favorable conditions for ischemia liver to survive. However, the deleterious consequences of cold ischemia may be attenuated by the composition of the refrigerating solutions of the conservation. The conservation liquids permit the reduction of cellular edema by restoring the extracellular acidosis, to limit the expansion of interstitial space when perfusing the organ and the reperfusion lesions due to accumulated free radicals. (7)

To improve the conservation solution of organs, we estimate the capacity of *Ulva lactuca* extract.

We began by testing the antioxidant activity (DPPH test) and anti-lipoperoxidant activity of *Ulva* extract. We compared plant results to Ascorbic Acid and α Tocopherol the two antioxidants reference. The extract of *Ulva lactuca* shows low antioxidant power compared to Ascorbic Acid and α Tocopherol. And plant extract can't inhibited the lipid peroxidation damage (in vitro test). Then, we quantified the total phenols concentration of *Ulva lactuca* that shows low content. Previous studies have shown that phenols have antioxidant activities, which contribute to the explanation of the protective effect of plants against some diseases (13). The capacity of extracts to scavenger free radicals can be attributed to phenolic acids (14). The low antioxidant effect of *Ulva lactuca* is due to the low concentration in polyphenols. In spite of the low antioxidant, anti-lipoperoxidant powers and the low phenols concentration, we chose *Ulva lactuca* because of these properties described in the literature. Indeed, various studies showed that Ulva lactuca is rich in steroids with anti-inflammatory and antibacterial capacities (15), as polysaccharides resulting from the sea lettuce showed an anti-viral activity, as capacities anti-peroxydative and anti-hyperlipidimique (16). And also rich in varied Vitamins (B, C and A), in calcium and magnesium, in Amino acids (proline and lysine), in protein elastic Fibers, iron, zinc, and sodium, has us to lead to estimate its effect on the cold ischemia (15).

Although transplantation is the most effective treatment for end-stage liver disease, the duration of cold ischemia before graft remains a major source of morbidity. Poor initial graft function depends on the length of cold storage (17). Several hours of cold ischemia induce injury mediated by several intricate factors: increased cellular Ca^{2+} content (18), mitochondrial dysfunction (19) and decrease adenosine tyrosine phosphate (ATP) content (20).

The second part of this study, we aimed to find solutions to protect liver against lesions induced by cold ischemia, using *Ulva lactuca* extract.

To clarify the pathogenesis of ischemic liver injury, histopathological changes in the liver and liver function tests before and after ischemia were examined in rats. Liver MDA levels, a marker of oxidative stress, permitted us to obtain a quantitative estimation of the membrane lipid oxidative damage (21). In the present study, cold ischemia caused significant increases in the hepatic MDA levels, end products of lipid peroxidation. After 24 hours of conservation (solution of Krebs + *Ulva lactuca* extract), the algae extract have protected the hepatic mitochondria against the lipid peroxidation by 26.43% relative to non-treated group (table 4). Our results demonstrated that *Ulva lactuca* extract can inhibit the production of oxygen radicals and prevent them from injury to liver tissue. Certain authors showed that *Ulva lactuca* decreased the oxidative stress by increasing the activities of antioxidant enzymes (CAT, GSH-Px and SOD) and limiting lipid peroxidation process (22).

Our objective was to preserve mitochondrial respiration by utilizing natural compounds presented in *Ulva lactuca*. Such the percentage of improvement of respiratory activity with the addition of conservation solution reached 38.8% shown in the non-treated group (table 4). We showed that *Ulva lactuca* protects mitochondria and cells against cold ischemia. This protection is clearly seen on mitochondrial respiratory parameters. *Ulva lactuca* improved mitochondrial coupling after 24 hours of cold ischemia as demonstrated by the increase RCR values. The obtained results were correlated with MDA test.

Our study shows that the mitochondrial respiratory chain is highly affected after 24-h conservation and that the capacity of ATP production is decreased. Moreover, the mitochondrial membrane damage (increase of MDA after 24 h conservation) reduces the capacity of mitochondria to maintain the electrochemical gradient (decrease of V3 state). These alterations are at least in part due to the occurrence of PTP. Indeed, it is well established that PTP opening renders the inner membrane permeable to ions and solutes and thus induces swelling (23).

The study of mitochondrial respiration is then a complement test of dosing MDA, it partially informs the level of mitochondrial membrane protection against the lipid peroxidation effect and evaluates the activity of respiratory chain. Therefore, a good protection allows the decrease of calcium influx, which assure a cytoprotection in addition of inhibiting the ionic voltage dependent channels to limit calcium massive influx and ensure the activation of numerous dependent calcium enzymes (24). Hypothermic ischemia induced extensive damage to liver cells, as measured by the aminotransferase leakage, a good indicator of structural membrane damage (25).We noticed that the plasmatic level of the AST and the ALT increased after the ischemic phase. The addition of *Ulva*

lactuca extract in the preservation solution induced a decrease in ALT (94.92%) and AST (72.3%) activities (table 4). The transaminases release after ischemic phase is due to a cellular suffering (26). Ischemia causes a fall of the intracellular content of ATP and an accumulation of Ca^{2+} intracytoplasmic. The accumulation of calcium during ischemia will support the way of synthesis of the uric acid with depends on the way of formation of the ATP and thus a simultaneous appearance of necrosis and the apoptosis cells.

Cells usually soak in an extracellular environment rich in sodium (with calcium and chloride) and poor in potassium which is the main intracellular cation. As the potassium establishes a wide fraction of all the cellular solutions, it is a major determiner of the volume of the cell and the osmolarity of liquids of the body. When the pump does not work, we attend a passive leak of the potassium in extracellular environment eliminating the gradient and in an equivalent entrance of sodium to the cell accompanied with the ions chlorinate (the passive distribution is not affected by the hypothermia) (27).

This creates an increase localized by the intracellular osmolarity entraining then a movement of water, because the intracellular anions not diffusible (proteins and organic phosphates) cannot leave the cell to counterbalance this effect. Indeed, to maintain the osmotic balance, the massive entrance of sodium to the cell comes along with a massive entrance of water, which forms a cellular inflation, then a cellular edema (28). The dosage of K^+ in the conservation solution after 24 hours of hepatic ischemia has revealed a significant difference between non treated livers (Krebs) and treated livers (Krebs + extract). This is shown a decrease in cellular lyses. The dosage of Ca^{2+}, Cl^- and Na^{2+} in the solution of conservation and after 24 hours of hepatic ischemia didn't show significant difference between groups.

Cold ischemia injury induces cell death through apoptosis and necrosis (29) or a combination of both called necroapoptosis (30). The present study clearly demonstrates histopathologically that ballooning degeneration of the liver cells developed during ischemia is the primary lesions of the ischemic liver injury. Consequently, this will results in intracellular accumulation of Na^+ and K^+ diffusion outside the cell.

It is also noted the appearance of apoptotic cell after 24 hours of cold ischemia. Indeed, apoptosis is involved in liver cell damage during organ preservation. It has been shown that hepatocytic apoptosis plays an important role in cold-preserved liver transplantation. Thus, inhibition of apoptosis and ballooning degeneration (reversible phase of necrosis) seems to be a rational strategy to reduce the risk of ischemia injury in liver drafts. These lesions disappear in the group treated with *Ulva lactuca* extract.

In conclusion, *Ulva lactuca* protects hepatocytes from the deleterious effect induced by hypothermic ischemia in rat model. This cytoprotective effect was characterized by a reduction of the leakage of hepatic enzymes, a reduction of histological damages and an improvement in liver metabolic capacities.

Conflict of Interest

The authors declare no conflict of interest.

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