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Evaluation The synergism Activity of *Portunus armatus* and *Apium graveolens* Extract as Antioxidant in Rats Exposed to Oxidative Stress

Wasan Sarhan Oubeid
Department of Physiology and Pharmacology and Biochemistry, Collage of Veterinary Medicine,Tikrit University, Tikrit, Iraq.

THE *Portunus armatus* is a crab that is highly valuable economically, has a broad geographic distribution and is used medicinally. It is also regarded as seafood in several countries like Iraq. Celery (*Apium graveolens*) is a native medicinal plant to Europe. This plant has a Very wide range of usage and cultivation. Celery (*Apium graveolens*) is usually used in traditional medicine as a diuretic or anti-hypertensive agent.

The study included 15 experimental rats aged 2-3 months, weight 150-250gm .all the experiments were done in animal house/the college of veterinary medicine at the university of Tikrit. The 15 rats disterbured into 5 groups (G1 given distilled water for four weeks as a negative control group, G2 given hydrogen peroxide at concentrations 1% with distal water for four weeks as a positive control, orally using gavage,G3 given hydrogen peroxide at concentrations 1% with *Apium graveolens* extract of one ml for each rat daily, G4 given hydrogen peroxide at concentrations 1% with *Portunus armatus* extract 1 ml for each animal daily and G5 given hydrogen peroxide at concentrations 1% with given the both extract daily (1ml of ach one) for four weeks, respectively. At the end of experiment the animals were scarified. The blood was drawn by orb, to obtain the blood for hematological tests and the serum for biochemical tests,

The levels of MDA increased significantly in the G2 comparative with control group and others treatment group's p ≤ o.o5, The TAC and Glutathione peroxidase level decrese significantly in oxidative stresses group G2 P ≤0.01 and increase in alone extract comparative with the other groups, while the level of TAC increase significantly in synergism group comparative with oxidative stress group (p≤ 0.05).

the concentration of SOD reduced in oxidative stress comparative with control group (p≤0.05), while its return to normal value in the synergism group pf both extract.

The results show a significant increase of liver enzyme and glucose level in oxidative exposure animals comparative to control group were the enzymes began to return to its normal value after treatment with both extract. High significant (p≤ 0.01) was obtained through the synergism effect.

Key words: *Portunus armatus* , *Apium graveolens,* oxidative stress, oxidative markers, synergism effect.

Introduction

The name crustacean, which derives from the Latin crusta, which means shell, refers to all members of the phylum Arthropoda Crustacea [1]. The invertebrate group known as crustaceans is incredibly diverse and includes both sessile and active organisms, such as crabs, lobsters, prawns, krill, copepods, and amphipods. One of the most Animal Kingdom's largest phylum is Arthropoda, which It includes about11,340,000 species in all habitats [2] Approximately 83% of all animal species on Earth are represented by this. Arthropoda encompasses a wide variety of

*Corresponding author: Wasan Sarhan Oubeid, E-mail: ewiesfawzy@yahoo.com, Tel.: 009647708006324
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insects, such as spiders, scorpions, prawns, crabs, millipedes, and centipedes. [3]

The Blue Swimming Crab (*Portunus armatus*) is one of the most important commodities in fisheries in the world, and crabs are valuable seafood products, both at home and for export, and therefore many countries have exploited sea crabs to support their economies [3,4]

Worldwide, coastal and estuarine areas are frequently inhabited by the diverse family of crabs known as Portunidae, Portunid crabs play a vital role in commercial fisheries around the world, with the Indo-West Pacific seeing the majority of landings. [4]

Additionally, they support significant recreational fisheries in nations like Australia and the United States and are fished on a smaller scale for subsistence and to supplement income in underdeveloped nations. [5; 6]

Crab meat is characterized by its low calorie content, and most of these calories come from the proteins in it, which contribute to building muscles, skin and bones, it is worth noting that it also contains many useful nutrients, such as: Minerals [7]: Crab contains some minerals in abundance, including: Zinc: helps the body makes use of proteins, carbohydrates, and fats in food after eating it, and also contributes to wound healing [8] Copper: helps the production of white and red blood cells, stimulates the release of iron to produce haemoglobin, in addition to its importance for the growth of infants, the development of the brain, the immune system, and bones. Selenium: Selenium plays an important role in the body's antioxidant defines system, thus reducing the risk of tissue and cell damage [9]. Magnesium: which contributes to the process of converting food into energy, and helps the glands to secrete hormones important for bone health, and it should be noted that lobster contains magnesium in a good amount, but it is not as much as other minerals[4,10].

The Apiaceae family includes *Apium graveolens,* also known as celery. In regions of Africa, Asia, and Europe with tropical climates, celery plants can be found [10]. The seeds, stalks, leaves, and stems of celery can be used to cure gout, rheumatism, urinary tract inflammation, and arthritis (although up to this point, celery has been consumed and grown all over the world) [11] Celery can also be used as a diuretic, for stimulation of the glands, bile, kidney stones, to regulate the intestines, to increase appetite, and as a prophylaxis for nerve agitation [12]

The reactive oxygen species (ROS) are created by biological organisms as a byproduct of regular cellular metabolism. At low to moderate concentrations, they are useful for physiological cell processes, but at high concentrations, they can negatively alter the lipids, proteins, and DNA of cells [13] "Oxidative stress" refers to the shift in the antioxidant/oxidant balance in favour of oxidants. Numerous pathogenic illnesses, such as cancer and neurological disorders, are exacerbated by oxidative stress, hypertension, atherosclerosis, and ischemia/perfusion [14] other diseases can be resulting from imbalance of oxidants/anti-oxidants such as: "Diabetes, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease acute respiratory distress syndrome, and asthma" [15]. Aerobic living organisms have developed antioxidant systems that often work well to counteract the negative effects of ROS, these systems include both enzymatic and non-enzymatic antioxidants. However, pathological situations can overload the antioxidant mechanisms [16].

This study aimed to know the synergism effect of both *Portunus armatus* and *Apium graveolens* extract against oxidative stress.

Methodology

Animal distribution

Experimental rats aged 2-3 months and weights 250 gm were used in this study. The 15 rats divided into 5 groups (G1 given distilled water for four weeks as a negative control group, G2 given hydrogen peroxide at concentrations 1% with distal water for four weeks as a positive control, orally using gavage,G3 given hydrogen peroxide at concentrations 1% with *Apium graveolens* extract at concentration 150mg/kg.bw for each rat daily, G4 given hydrogen peroxide at concentrations 1% with *Portunus armatus* extract at concentration 250mg/kg b.w for each animal daily and G5 given hydrogen peroxide at concentrations 1% with given the both extract daily (150, 250 mg/kg bw) for four weeks, respectively.

Extracting the Portunus armatus shell

The swimming blue crab was obtained from

the local markets of the city of Basra, where it was transported in refrigerated containers to ensure that it was not damaged or infected with any pollutants. In the beginning, the animals were washed with sterile water and sterile salts and left to dry, and then the shell was separated from the rest parts, where the legs and other body appendages were removed.

The shell from the inner well was cleaned, and it was allowed to dry at room temperature for a week in the lab. After creating a fine powder out of it, 25g of the powder was taken and diluted with distilled water by 25%. [17]

Extraction of Apium graveolens

The celery plant was taken and dried at room temperature for one week, then ground into a fine powder, then it was extracted by means of the saxolites device by taking 25 grams of the powder with 100 ml ethanol, putting the extract after the completion of the extraction process by the device, in glass jars (Pyrex) and waiting for two weeks to dry, then 10 gm of extract obtained and solvent in 90 ml of distilled water for using.

The blood sample preparation

The blood samples were obtained from orb and placed in a clean, simple vacutainer before using at the biochemistry lab. The sample was immediately centrifuged for five minutes, and the clear serum was kept in Eppendorf tubes at 60° C.

Oxidative stress markers

Serum Glutathione peroxidase level

550 mL of phosphate buffer containing ethylenediaminetetraacetic acid, 50 mL of sodium azide, and 100 mL of glutathione reductase were added together with 50 mL of serum. After this step the solution incubate for 10 minutes at 37 °C. Nicotinamide adenine dinucleotide phosphate 100 L was then added to the solution. By adding 100 L of H_2O_2, the reaction was noticed. The optical density was measured for five minutes at 340 nm at 1-min times. Thus, glutathione peroxidase's activity was recorded [18].

Malondialdehyde level

Malondialdehyde (MDA) level was calculated using the Yagi technique [19] and represented as _mol/L. The outcomes were computed using an MDA absorption coefficient.

Total antioxidant capacity level

FRAP assay was used to determine total antioxidant capacity in accordance with [20] technique. Low pH results in the reduction of a ferric tripyridyltriazine (Fe III-TPTZ) complex to the ferrous (Fe II) form, which results in the development of a strong blue colour with a maximum absorption at 593 nm.

Super oxide dismutase SOD

SOD enzyme activity was measured using a spectrophotometric technique using KO_2 in accordance to [21] approach, with a few alterations. Units per milliliter of serum are used to present the data.

Determination of liver function testes (ALP, AST, and ALT)

Colorimetric determination of ALP, AST and ALT was used by a kit supplied by biomerieux company, France. The results were obtained by spectrophotometer at read absorbance 510 and 450, respectively.[22]

Blood glucose estimation

Enzymatic estimation of fasting blood glucose (FBS) was performed in ,accordance with the company" Randox GOD/PAP-U.S.A.'s instructions".

Statistical analysis

Each result was signified as mean ± standard deviation. the data Statistical significance was determined by Student's t-test, and one-way ANOVA at a significance level of 5%. SPSS version 16.0 was using to preform Statistical analysis of study data. [23]

Results and Discussion

Antioxidant marker

In total, 15 male rats from the control and treatment groups were used in the present research. Table 1 shows the findings of the measurement of oxidative stress indicators in the entire treatment group in comparison to the control group.

The levels of MDA increased significantly in the oxidative stress state comparative with control group and others treatment groups $p \leq o.o5$, the level of serum MDA reduce significantly in the last group of both extract of *Apium graveolens* and *Apium graveolens*. MDA It is an indicator of oxidative stress as it creates an end product of the lipid oxidation chain reaction in order to prevent oxidative stress [24]Omega-3 and omega-6 fatty acids' carbon double bonds are attacked

by free radicals during a process known as lipid peroxidation, which also produces reactive aldehydes like MDA as a by-product [25].

Glutathione peroxidase and TAC level decrees significantly in oxidative stresses group which is treated with H_2O_2 P ≤0.01 comparative with the other groups, and also decrease (P≤0.05) in alone extract comparative group, while the level of TAC increase significantly in synergism group comparative with oxidative stress group (P≤0.05).

The results indicated that the concentration of SOD reduced in oxidative stress comparative with control group (P≤ 0.01), while its return to normal value in the synergism group of both extracts (P≤0.05).

Glutathione peroxidase performs many different functions in the cell, including that it plays an important role in the removal of toxins and heavy metals, and in the reduction of some reactive oxygen species (ROS), [25] for example hydrogen peroxide, This study revealed a significant anti-oxidant effect of ethanol extract of *Apium graveolens* and shell extract of *Portunus armatus* in experimental models of rats exposed to oxidative status.

Only by measuring the overall antioxidant capacity of the organism can the precise antioxidant capacity of the organism be ascertained. The FRAP assay is "described as a novel technique for determining total antioxidant capacity and is thought to be a valuable barometer of the body's antioxidant status" to prevent oxidative damage brought on by ROS [26] .

Despite oxidation processes are crucial for cells to function, they can also cause damage. As a result, animals as well as plants have a variety of antioxidants, including the vitamins C and E and glutathione. They also have various enzymatic systems that catalyse the antioxidant reactions, such as catalase, superoxide dismutase (SOD), and peroxidases. [27] Oxidative stress brought on by deficiencies in or inhibition of these antioxidant enzymes may cause cell damage and lysis [28] The processes that antioxidant defense uses are as follows: inhibiting the generation of oxidants and free radicals scavenging, turning harmful free radicals into less harmful compounds, "Blocking the production of secondary toxic metabolites and mediators of inflammation, Blocking of the chain propagation of the secondary oxidants , Repairing the injured molecules , Initiation and enhancing the endogenous antioxidant defence system" [29]. To protect the organism from oxidative stress, each of these defence mechanisms works in concert with the others. Animal and human antioxidant systems are made up of potent enzymatic and non-enzymatic antioxidants [30].

Antioxidant compounds are chemical compounds that help suppress free radicals or inhibit them from reacting [30], to guard against the negative effects caused by free radicals, cells have sufficient defensive hosts. The enzymes superoxide dismutase SOD and glutathione peroxidases GPx are examples of antioxidants [31].

Numerous phenolic chemicals found in celery make it a potent source of antioxidants [32]. The radical activity of 1,1-diphenyl-2-picrylhydrazyl was used to study the antioxidant activity of celery leaves. It is related with other antioxidant substances such as L-tryptophan and derivatives of methoxy-phenyl chromenone, and is recognised to be a natural antioxidant by blocking the oxidative process [33] .

"Celery stems and seeds can be used as anti-inflammatory, hypotensive, carminative, urinary antiseptic, sedative, antirheumatic, and spasmolytic antiseptic" [34].

Chitin (20–30%), protein (30–40%), calcium carbonate salts (30–50%), and antioxidant substances like selenium and carotenoids (Astaxanthin, Astatin, and Xanthine) are the primary natural components found in the shells of crustaceans like *Portunus armatus* [35] .

Crab shell extract inhibits breast cancer cell line proliferation in a dose- and time-dependent manner [36]. The extract appears to exert its effect by reducing NOx production and inducing apoptosis, as chitin, carotenoids and selenium derivatives are important factors that must be considered in the growth inhibition process In addition, it is a great source of several vitamins (B2, B3, B12, and C) and minerals (such as iron, calcium, potassium, and phosphorus), all of which assist in avoiding oxidative damage to cells and tissues and serve as antioxidants by counteracting the effects of carcinogens [37].

A review of the literature found that the waste from carb shells is a rich source of phenolic chemicals. Phenolic chemicals were

also discovered to have a variety of biological effects, including antioxidant, antibacterial, anti-inflammatory, and anticancer activity. Phenolic compounds play a significant part in the ant oxidative characteristics [38].

The results in Table (2) showed significant increase ($p \leq 0.05$) ($p \leq 0.01$) in mean level of ALT, AST, and ALP enzymes of group exposure to oxidative stress, comparative to control group respectively, this increasing start to decreasing in the groups treated with *apium graveolens* and *portunus armatus*.

The increase in liver enzymes may be caused by damage to tissue inflicted on the liver as a result of oxidative stress brought on by H_2O_2 exposure, as well as by the adverse impacts of the metabolites. The leakage of these enzymes from the liver cytosol into the blood stream as a result of tissue injury was the primary cause of the increase in the level of these enzymes [39].

This result was in agreement with previous studies which showed that H_2O_2 caused an increase in liver enzymes activity, who reported that Liver damage brought on by oxidative stress is characterized by an increase in lipid peroxide activity, a decrease in glutathione levels, and an increase in superoxide dismutase activity. [40]

Increases in ALT, AST, and ALP are linked to liver injury and disease, while inadequate antioxidant defences are linked to elevate this enzyme, especially low glutathione levels [41].

The observed decrease in AST and ALT may point to the antioxidant and hepatocyte-protective properties of celery and blue carb extracts. The reason for the low level of liver enzymes in the groups treated with blue crab powder extract compared to the H_2O_2 group is due to the effectiveness of the blue crab powder extract in removing the harmful effect of free radicals, and this is consistent with .This study showed that treating male rats with crab shell extract led to an increase in endogenous antioxidant.

The results of Table (2) showed a significant increase ($P \leq 0.05$) in the blood glucose concentration in oxidative stress group, and a significant decrease in blood sugar in groups that treated with the both extract, which are treated with *Apium graveolens* and *Portunus armatus* extract as compared to the control group. The result of the decrease in glucose in the *Portunus armatus* group is in agreement with [, as treatment of male rats with crab shell extract reduced the blood glucose concentration. Previous study Show that eating crab meat lowers blood sugar. As they reduced the elevated blood glucose levels in rats, celery extract and blue carb extract both had positive effects on how carbohydrates are metabolised.[42]

The significant increase in blood sugar in oxidative exposure group is consistent with previous study as the treatment of male rabbits with hydrogen peroxide led to a significant increase in the level of glucose compared to the control group [43]. Due to an increase in the production of various ROS and nitrogen free radicals, this rise may be linked to a complication with the insulin secretion from the pancreatic beta cells. Or, it could be brought on by an increase in the hormone concentrations that accelerate the conversion of glycogen-6-phosphate during the process of glycolysis, such as adrenaline.

Conclusion

The using of seafood nowadays is one of the most important sources of substances and nutrients beneficial to the body because it contains rich sources such as minerals and vitamins, which play an important role in the antioxidant system and the defense against free radicals. Vegetables are also an important source of many nutrients that the organism needs, all of which act to face Cell damage enhance body functions. This has been proven by the current study.

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Conflict of interest: None

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TABLE 1. Effect of *Portunus armatus* and *Apium graveolens* extract on oxidative markers in rats exposed to H_2O_2.

Groups / Treatments	MDA(µmol/L)	Glutathione peroxidase U/L	TAC(µmol TE/L)	SOD(units/ml)
Control	1.69± 0.31	11.32±0.9	913.68±16.72	8.75 ± 1.1
Oxidative group (H_2O_2)	4.22± 0.8*	7.56 ± 2.7**	539.56±22.4**	4.9 ± 0.4**
Portunus armatus extract	3.91 ± 0.4b*	8.89±1.5*	374.34±12.6**	6.12±0.6**
Apium graveolens extract	2.66± 0.7	9.22±1.2*	328.25±11.2**	6.19±0.7**
Portunus armatus extract and *Apium graveolens* extract	2.21±0.2	9.59±0.4*	822.61±8.36*	7.8±3.2*

*Significant differences at p≤0.05
** Significant differences at p≤0.01

TABLE 2. Effect of *Portunus armatus a*nd *Apium graveolens* extract on liver markers indices in rats exposed to H_2O_2

Variables / Groups	ALT IU/L	AST IU/L	ALP IU/L	Glucose mg/dl
G1	46.23±4.365*	33.32±2.85	85.58±4.03	96.32±3.88
G2	69.58±3.32**	48.25±2.58*	120.67±5.98**	120.63±3.88*
G3	55.84±5.57	41.23±2.89*	99.35±4.36**	108.36±1.34**
G4	54.41±3.24	43.29±3.56*	91.58±7.98**	98.65±1.74

*Significant differences at p≤0.05
** Significant differences at p≤0.01

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تقييم النشاط التآزري لمستخلص *Portunus armatus* و *Apium Graveolens* كمضاد للأكسدة في الجرذان المعرضة للإجهاد التأكسدي

وسن سرحان عبيد

فرع الفسلجة والادوية والكيمياء الحياتية - كلية الطب البيطري - جامعة تكريت - تكريت - العراق.

Portunus Armatus :هو احد انواع سرطان البحروهو ذو قيمة اقتصادية عالية، وله توزيع جغرافي واسع ويستخدم في المجالات الطبية. كما يعتبر من المأكولات البحرية في عدة دول مثل العراق. الكرفس (Apium Graveolens) هو نبات طبي منشاه الاصلي في أوروبا. هذا النبات لديه مجموعة واسعة جدا من الاستخدامات وكذلك في مجال الزراعة. يستخدم الكرفس (Apium Graveolens) عادة في الطب التقليدي كعامل مدر للبول أو مضاد لارتفاع ضغط الدم.

شملت الدراسة ١٥ فأراً تجريبياً بأعمار ٢-٣ أشهر وأوزان ١٥٠-٢٥٠ غم. أجريت جميع التجارب في البيت الحيواني/كلية الطب البيطري في جامعة تكريت. تم تقسيم الفئران الخمسة عشر إلى ٥ مجموعات (G1 أعطيت الماء المقطر لمدة أربعة أسابيع كمجموعة ضابطة سلبية، G2 أعطيت بيروكسيد الهيدروجين بتركيزات ١٪ مع الماء المقطر لمدة أربعة أسابيع كمجموعة ضابطة إيجابية، عن طريق الفم ، G3 أعطيت بيروكسيد الهيدروجين بتركيزات ١٪ مع مستخلص Apium Graveolens بمقدار مل واحد لكل فأر يومياً , ، G4 أعطيت بيروكسيد الهيدروجين بتركيز ١٪ مع مستخلص Portunus armatus1 بمقدار ١ مل لكل حيوان يومياً وG5 أعطى بيروكسيد الهيدروجين بتركيز ١٪ مع إعطاء المستخلصين يومياً (١ مل من كل مستخلص) لمدة أربعة أسابيع على التوالي. في نهاية التجربة تم التضحية بالحيوانات. تم سحب الدم عن طريق محجر العين، للحصول على الدم لإجراء اختبارات الدم والمصل للاختبارات البيوكيميائية، ارتفعت مستويات MDA بشكل ملحوظ في مجموعة G2 مقارنة مع مجموعة السيطرة ومجموعة العلاج الأخرى p ≥ o.o5، كما انخفض مستوى TAC و الجلوتاثيون بيروكسيديز بشكل ملحوظ في مجموعة الاجهاد التأكسدي G2 P≥0.01 وزيادة معنوية في مجموعة المستخلص وحده مقارنة بالمجموعات الأخرى. بينما ارتفع مستوى TAC بشكل معنوي في مجموعة التآزرية مقارنة بمجموعة الإجهاد التأكسدي (p≥0.05).

انخفض تركيز SOD في الإجهاد التأكسدي مقارنة بمجموعة السيطرة (p<0.05)، في حين عاد إلى قيمته الطبيعية في مجموعة التآزر في كلا المستخلصين.

أظهرت النتائج وجود زيادة معنوية في مستوى إنزيمات الكبد وسكر الدم في الحيوانات المعرضة للتأكسد مقارنة بالمجموعة الضابطة حيث بدأت الإنزيمات بالعودة إلى قيمتها الطبيعية بعد المعاملة بكلا المستخلصين. تم الحصول على معنوية عالية (p<0.01) من خلال التأثير التآزري.

الكلمات المفتاحية : مستخلص الكرفس، مستخلص سرطان البحر, الإجهاد التأكسدي، مؤشرات الأكسدة, التأثير التآزري.