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Evaluation the effects of blue crab Shell extract on some hematological and biochemical parameters in male rats induced cyclophosphamide.

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ABSTRACT

The aim of this study was carried out to determine whether crab Shell extract (CSE) has potential therapeutic and preventive effects when administrated (500 mg\Kg) once daily for four weeks would prevent cyclophosphamide-induced hepatotoxicity in albino male rats. Methods: Total of twenty-five male rats were divided randomly into five groups of 5 rats each as follows: negative control group (NC), positive control (PC) group, Normal + CSE, positive control+CSE (PC+CSE) and the Preventive group + CSE. Follow four weeks of treatment Total Protein (TP), liver enzymes include Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatases (ALP) and complete blood count (CBC) were measured. Results: There were significantly different in the values of MCV, MCH, MCHC, RBC, WBC, Plt and Hb among all study groups when compared with NC group. PC group had a significantly increased AST level compared to the normal group, and a significantly decreased of AST level among normal+CSE compared to CP+CSE and Preventive group + CSE P=0.003, P= 0.001 respectively. our results also showed that ALT levels were significantly higher ($p<0.001$) in CP group as compared to CP+CSE group. Decreased levels of ALP ($P<0.001$), were observed in CP+CSE group when compared to the positive control. on the other hand, there were no significantly different in the total protein among all groups of study. Conclusion: Four weeks of daily supplementation with 500mg\kg of crab Shell extract (CSE) was found to be able significantly improve biochemical and hematological changes caused by cyclophosphamide.

تقييم تأثير مستخلص قشور السلطعون على بعض المؤشرات الدموية والبيوكيميائية في ذكور الفئران المستحدثة بعقار السيكلوفوسفاميد

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الكلمات المفتاحية:
السيكلوفوسفاميد
قشور السلطعون
التغيرات الدموية
انزيمات الكبد
مضادات الاكسدة
ذكور الجرذان

الملخص

الهدف من هذه الدراسة هو تحديد ما إذا كان مستخلص قشرة السلطعون (CSE) الذي ثبت أن له تأثيرات علاجية ووقائية محتملة عند تناوله (500 مجم / كجم) مرة واحدة يوميًا لمدة أربعة أسابيع سيمنع السمية الكبدية التي يسببها سيكلوفوسفاميد في الجرذان البيضاء. تم تقسيم خمسة وعشرين من ذكور الجرذان بشكل عشوائي إلى خمس مجموعات من 5 فئران لكل منها على النحو التالي: مجموعة التحكم السلبية (negative control) ، مجموعة التحكم الإيجابي (positive control) مجموعة Normal + CSE ، مجموعة PC + CSE والمجموعة الوقائية ± CSE . بعد أربعة أسابيع من العلاج ، تم قياس إجمالي البروتين (TP) ، وأنزيمات الكبد تشمل Alanine Aminotransferase (ALT) ، و Aspartate

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Aminotransferase (AST) ، و Alkaline Phosphatases (ALP) وتعداد الدم الكامل (CBC). النتائج: كانت هناك اختلاف كبير في قيم MCV و MCH و MCHC و RBC و WBC و Plt و Hb بين جميع مجموعات الدراسة عند مقارنتها مع المجموعة الطبيعية. أظهرت مجموعة التحكم الإيجابي زيادة كبيرة في مستوى AST مقارنة بالمجموعة الطبيعية، وانخفاض ملحوظ في مستوى AST في مجموعة normal+ CSE مقارنة بـ CP + CSE والمجموعة الوقائية + CSE P = 0.003، P = 0.001 على التوالي. أظهرت نتائجنا أيضًا أن مستويات ALT كانت أعلى بشكل ملحوظ (p <0.001) في مجموعة CP مقارنة بمجموعة CP + CSE. لوحظ انخفاض مستويات ALP (P <0.001) في مجموعة CP + CSE بالمقارنة مع السيطرة الإيجابية. من ناحية أخرى لم يكن هناك اختلاف كبير في البروتين الكلي بين جميع مجموعات الدراسة الخلاصة: تناول مستخلص قشور السلطعون بجرعة 500 ملجم / كجم يوميا لمدة أربعة اسابيع من قادرة على تحسين التغيرات الكيميائية الحيوية والدموية الناتجة عن عقار السيكلوفوسفاميد

Introduction

Cancer is aberrant cell proliferation brought on by critical gene alterations. Oncogenes, tumor suppressor genes (TSG), DNA repair genes, as well as apoptotic and apoptotic genes, are important genes. The invasion of cancer cells into the body's various organs results in tumors, and the deformation of organs causes death [1] Neoplasms and malignant tumors are other words that are used. The rapid development of aberrant cells outside of their normal borders, invasion of other body parts, and ultimately spread to other organs are all traits of cancer. The term "metastasis" refers to this action. Widespread metastases are thought to be the primary cause of death in cancer patients [2] . It is responsible for approximately 1 in 6 deaths worldwide[3]. by 2030 13.1 million deaths is expected due to cancer-causing infections [2,3];. Various techniques can be used to treat cancer, such as radiotherapy, surgery, immunotherapy, gene therapy, and chemotherapy. The most common method of treating cancer is still chemotherapy. Currently utilized cancer treatments come with unpleasant side effects, poor effectiveness, and expensive costs[2]. Cyclophosphamide, one of the most efficient chemotherapy drugs, is included among the essential treatments by the World Health Organization. Cyclophosphamide is widely used in the treatment of various neoplastic diseases and diseases associated with altered immunity. Using more CP for a longer period of time can have negative consequences on several organs, including the heart, liver, lungs, and male reproductive organs. [4].

According to the classification of anti-cancer medications, the medication is grouped with alkylating agents[5, 6]. Despite many side effects of this drug, such as hair loss, nausea, vomiting, skin color change, burning sensation during urination, loss of appetite or weight , as well as the appearance of pathological tissue changes in the kidneys and Liver, blood, bladder, and jejunum[7, 8]. Based on previous studies, the kidneys and liver had pathological alterations as a result of the medication of cyclophosphamide. treatment of cyclophosphamide causes toxicity in the liver, which is manifested by the development of necrosis, fatty alterations, fibrosis, and blood vessel damage. Additionally, it disrupts liver processes, which changes how the drug is metabolized inside the liver and increase liver enzyme levels [9]The most significant source of bioactive chemicals and medications comes from natural marine products. Natural remedies, which are chemical substances created by living things, have been utilized for many years to cure a wide range of illnesses. Humans have used natural products from many different sources over the years, including plants, animals, and microorganisms [10]. The first marine-derived compound was licensed in 1969, and more marine medicines were approved for the treatment of cancer. And 17 anti-cancer medications derived from marine chemicals have received approval as of 2022[11]. Crab shell extract has been demonstrated in numerous studies to have a variety of chemical components and chemicals with antioxidant and anti-inflammatory activities[12]. Along with protecting the liver, kidneys, and other body organs from toxicity brought on by drugs and antibiotics[13]. Based on these facts and due to the scarcity of studies that dealt with the importance of crab shell extracts in mitigating and preventing the harmful effects and oxidative stress caused by the chemical drug cyclophosphamide, which is widely used in hospitalization in our society, This study was designed to assess the potential therapeutic and preventive effect of the extract from crab shells on cyclophosphamide-induced hepatotoxicity in albino rats because there are no published reports on the effects of CSE on biomarkers of liver function and some haematological parameters

2. MATERIALS AND METHODS

Animal and diet

The Laboratory Animal Resources Unit, Faculty of Science, Sebha University Libya, furnished a total of 25 male Albino rats, which were employed. The animals were 200-350 g and 6–8 weeks old. Two rats were housed in plastic cages, and they were fed a typical diet of mouse pellets.

and unlimited access to tap water. The Animals were acclimatized to standard laboratory conditions (temperature 25 °C, with light-darkness cycles of 12 hour), for one week before commencement of the experiments and they had never been exposed to any chemicals before. The experimental was conducting in October 2022.

. The animals received complete care during the breeding and experimentation phases. Crab shells were used as well in this study because samples of the Blue Crab (Portunus segnis) were taken from the shores of Zora, a city in Libya that is situated along the Mediterranean Sea.

Injury creation:

Rats were given intravenous injections of cyclophosphamide at a dose of 5 mg /body weight daily for 7 days to cause the infection.

Preparation of alcoholic extract of blue crab shell (*Portunus segnis*)

Crab samples were collected, completely washed with tap water, then washed again with distilled water. They were then allowed to dry naturally before being pulverized in an electric mill. 400 milliliters of 70 percent ethanol were used to dissolve 20 grams of powdered crab shell. It was vibrating in an opaque box for 48 hours. The alcohol was then filtered through a piece of medical gauze and placed in a drying oven to guarantee that it evaporated. [14]

Experimental Design

Total of twenty-five male rats were divided randomly into five groups of five rats each as follows

1. negative control group (NC) received doses of normal saline for four weeks

2. positive control (PC) group was induced injection of CP (5 mg/kg body weight/day for 7 days, followed by a 21-day orally administrated daily doses of normal saline.

3. Normal + CSE group was administered orally Crab shell extract (CSE) at dose 500mg/kg body weight / day

4. PC+CSE group was induced injection of CP (5 mg/kg body weight/day for 7 days, followed by a 21-day orally administrated of Crab shell extract (CSE) at dose 500mg/kg body weight / day

5. Preventive group + CSE, received crab shell extract CSE at dose 500mg/kg body weight / day of for 21 days followed by injections of CP for 7 days.

Blood samples collection

All the rats were fasted overnight and sacrificed by cardiac puncture under deep anesthesia with diethyl ether. The amount of food and water intake was measured and the body weights of all rats were recorded once a week

Under deep anaesthesia, the blood of all rats was taken by cardiac puncture using 21 G needles with 5 ml syringe.
the collected samples were divided into two vacationer tubes. Two ml in ethylene diamine tetra acetic acid (EDTA) for complete blood counts analysis and other (three ml) of whole blood were put in plain tubes. Erythrocyte pellets were separated from plasma and the leukocyte layer by centrifugation in 4,000 rpm for 15 min at 4 °C. The plasma in supernatant was pipetted into 1.5 ml Eppendrof tubes and was kept prior to analysis at -80 °C.
Hematological Study.

Freshly collected blood samples were analyzed using an Sysmex (hematology analyzer) which was used for complete blood count. total erythrocyte, total white blood cells (WBCs), hemoglobin (Hb) concentration, haematocrit (Hct), mean cell hemoglobin (MCH), mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), erythrocyte distribution withed (RDW) and platelet (Plt) were considered to be measured directly**.**

Determination of Biochemical Profile

Enzymatic assay was used to quantify total protein and liver enzymes such as Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatases (ALP).

Statistical analysis:

All results were expressed as mean ± standard deviation (SD) in the tables and mean ± standard error (SEM) in the graphs. The data were analyzed using SPSS (Statistical package for social sciences) IBM version 20. Shapiro- Wilk test was used to check the normality of the variable. Accordingly, ANOVA, were used to analyze data follow normal behaviour of distribution pattern, followed by post hoc LSD multiple comparison test were used to estimate the significance different between groups. While, Kruskal-Wallis-one way ANOVA test were used to analyze data follow non-normal behaviour of distribution pattern. The difference between groups was considered significant when $p< 0.05$.

3. Results

1. **Effects of Crab shell extract (CSE) on liver enzymes**
   The level AST was significantly higher in positive control compared to negative control group. interestingly, PC±CSE group and normal ± CSE showed significantly decreased in the levels of AST when compared to positive control group (figure. 1).

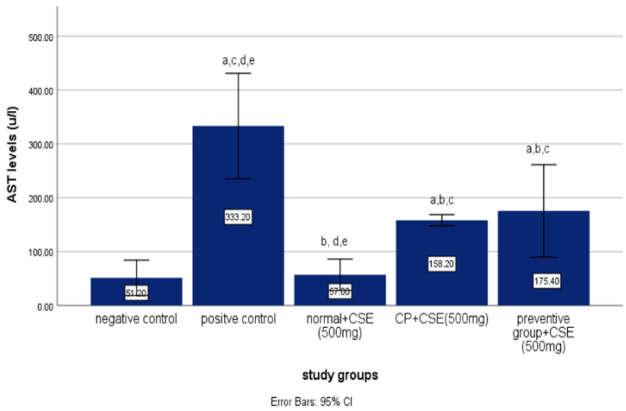

Figure 1. Effects of Crab shell extract (CSE) on AST levels. Results are expressed as the mean ± S.E.M.; (a) significantly different from the negative control (b) significantly different from the positive control (c) significantly different from the normal ± CSE group, (d) significantly different from the PC+CSE

Increased levels of ALT were observed in positive control compared to normal±CSE and PC±CSE p=0.001,0.0001 respectively. but CSE supplementation significantly decreased levels of ALT which was remarkable in PC±CSE figure 2.

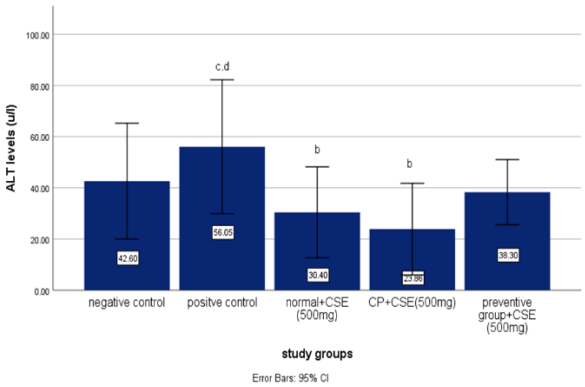

Figure 2. Effects of Crab shell extract (CSE) on ALT levels. Results are expressed as the mean ± S.E.M.; (a) significantly different from the negative control, (b) significantly different from the positive control , (c) significantly different from the normal ± CSE group, (d) significantly different from the PC+CSE

Plasma ALP was significantly higher in positive control group and normal ±CSE compared to the negative control rats, on the other hand CP±CSE showed significantly lower of ALP levels compared to positive control group alone figure 3.

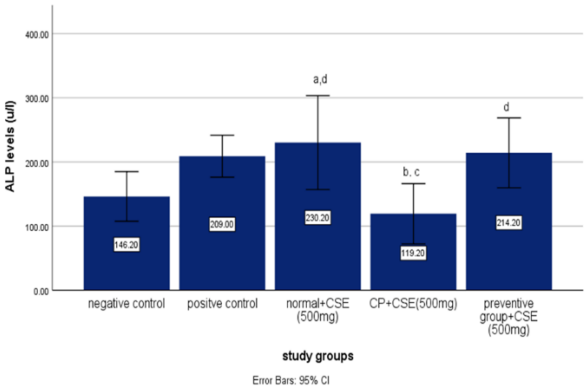

Figure 3. Effects of Crab shell extract (CSE) on ALP levels. Results are expressed as the mean ± S.E.M.; (a) significantly different from the negative control, (b) significantly different from the positive

control, (c) significantly different from the normal ± CSE group, (d) significantly different from the PC+CSE

as figure 4 showed there were no significantly different of total protein levels among all of study groups

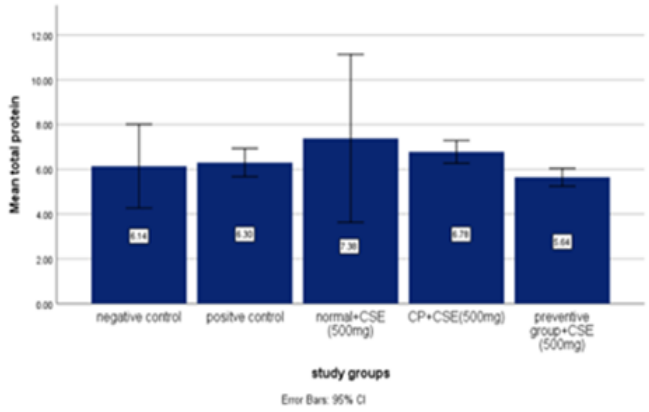

Figure 4. Effects of Crab shell extract (CSE) on total proteins levels. Results are expressed as the mean ± S.E.M.; (a) significantly different from the negative control, (b) significantly different from the positive control , (c) significantly different from the normal ± CSE group, (d) significantly different from the PC+CSE

2. Effects of Crab shell extract (CSE) on Hematological parameters

Table 1 shows the results of complete blood count. There were significantly different in the values of HGB, MCV, MCH, MCHC, RBC, WBC, Plt among all study groups when compared with NC group. The MCHC concentration was significantly lower in the positive control and Normal + CSE groups compared to the negative control group, and the percentage of Plt values in the CPCSE group and Preventive group+ CSE was significantly lower when compared to the negative control group (p values, 0.007, respectively). In contrast, there were no differences the percentage of PCV in all study groups

Table. 1 Effects of Crab shell extract (CSE) on hematological parameter

hematological parameters	**negative control**		**Positive Control**	**Normal+ CSE(500mg)**	**CP+ CSE(500mg)**	**Preventive group + CSE(500mg)**
RBC L×10^{12}	6.43±0.9		6.81±0.8	7.21±1.0*	6.78±0.94	5.85±0.8*
HGB **L/g**	13.02±0.5		12.26±1.2	14.9±1.9	12.45±1.23	12.06±0.93
MCV **(%)**	58.66±3.2		63.20±1.5	55.53±1.2*	57.67±2.2	64.62±12.5*
MCH	20.76±4.3		18.00±0.6	16.5±3.1*	18.42±0.7	20.45±2.09*
MCHC	34.78±5.9		28.40±0.9*	*29.70±5.4	32.02±1.9	31.88±1.5
WBC	5.30±3.1		6.10±1.3	6.86±3.4*	3.32±2.4*	4.53±1.2
PCV	38.08±4.8		43.14±5.0	40.06±5.9	39.25±7.0	37.08±3.7
PLT	490.4±145		429.2±72	582.8±107	244.5±68	268.8±141

Results are expressed as the mean ± S.E.M.; *****p < 0.05 compared to the normal group. RBC, red blood cells; WBC, white blood cells; Hb, hemoglobin concentration; Hct, hematocrit; MCH, mean cell hemoglobin; MCV, mean cell volume; MCHC, mean cell hemoglobin concentration; Plt, platelet

4. Discussion

In the current study, the effects of Crab shell extract on some of biochemical and haematological parameters in male rats induced cyclophosphamide were investigated. However, this investigation was carried out to determine whether 500 mg/kg BW gave the potential therapeutic and preventive liver damage. Several studies have been conducted to explore the anticancer and antioxidant effects of crab shell extract at various doses.

According to several studies, cyclophosphamide can harm multiple organs including liver, lung, spleen, kidneys and testes [14], [15],[16], [17]. the results of the current study, Cyclophosphamide was able to induce liver damage which remarkable by increasing AST, ALT and ALP levels among positive control group, these finding were in agreement with earlier studies which have been shown that Cyclophosphamide cause liver injury [18], [19] ،[20], which is consistent with the findings of the current study. interestingly, findings from the current study showed that Crab shell extract decreased levels of ALT ALP and AST in CP group supplemented with 500mg of CSE. which is consistent with the findings of the earlier study [21]

A traditional remedy for the treatment and prevention of cancer has been identified as crab shell, and studies have shown that its extract has potent inhibitory effects on the breast cancer cell line (MCF7) and the prostate cancer cell line (LNCap) [22, 23]. Different doses of extract improved liver tissue damages caused by cyclophosphamide. Crab Shell possesses a number of natural substances such as chitin and its derivatives, chitosan, chitin oligosaccharides, and chitosan oligosaccharide, with antioxidant agent (antioxidant compounds such as selenium and carotenoids [14]. According to reports, chitosan that has been isolated from crab shells exhibits a high antioxidant capacity, including the ability to chelate ferric ions and to block the generation of hydroxyl radicals [24]. Crab shell contains selenium and beta carotene with protective or inhibitory effects on oxidative stress [22], [23], Selenium is a component of enzyme catalyzed that redox reaction and acts as an antioxidant in the form of selenoproteins, thus, it is concluded that the extract can elevate antioxidant activity or decrease oxidative stress biomarkers[14] The results of the current investigation demonstrated a significant decrease in blood liver enzymes following the four-week administration of crab shell extract at a concentration of 500 mg/kg.

Regarding the findings of the whole blood count. There were significantly different in the values of MCV, MCH, MCHC, RBC, WBC among all study groups when furthermore, decreased levels of WBC were observed among CP ± CSE group compared with NC group. The lymphoid tissues are predicted to be more affected by cyclophosphamide [25]. Cyclophosphamide has an immunotoxic effect on blood and spleen lymphocytes in rats. Additionally, it has been noted that pretreatment of rats with cyclophosphamide significantly reduced the activity of all lymphoid cells, particularly CD4+ lymphocytes [26].

Therefore, the finding of this study indicated that rats treated with 500mg of crab shell extract showed an increased in hemoglobin and RBC. these finding are in agreement with previous studies that reported significant increased for these parameters [27]. It has been reported that crab shell extract are excellent source of vitamin A, vitamin B12, vitamin B6, thiamine and iron [28] These substances are known as the basic requirement needed for the production of RBC. The vitamins are capable of enhancing erythropoiesis and stimulating the maturation of the erythrocyte.

conclusion:

cyclophosphamide was able to induce liver injury which were remarkably observed by increasing liver enzymes, thus initial and follow-up liver function tests should be monitored in all patients receiving cyclophosphamide treatment. Crab shell extract considerably decreased the cyclophosphamide-induced biochemical and hematological alterations. Furthermore, four weeks of daily supplementation with 500 mg/kg of CSE was able to improve erythropoiesis, which was seen by a significant increase in Hg and RBC values, and reduce the levels of liver injury markers by decreasing liver enzymes. These findings imply that CSE may significantly mitigate the negative consequences of cyclophosphamide-induced rat impacts.These results suggest that CSE may have significant protective effects against the adverse effects of cyclophosphamide induced rats.

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