Chapter

Chemoprotective Effect of Edible Gastropod, *Xancus pyrum* and Its Usefulness in the Amelioration of Cisplatin Induced Toxicity

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Abstract

The main purpose of this study was to evaluate chemoprotective activities of methanolic extracts of an edible gastropod (*Xancus pyrum*) in cisplatin-induced immunosuppressed mice. Cisplatin (100 mg/kg, intraperitoneally [IP]) induced immunosuppressed mice were treated with a methanolic extract of *X. pyrum* (0.5 mg/dose/animal/IP) for a period of 10 days. The effect of the extract on lymphoid organ weight, bone marrow cellularity (BMC), alpha esterase activity, and on enzyme levels such as serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, urea, and creatinine was estimated to identify the chemoprotective activity of *X. pyrum*. The administration of *X. pyrum* extract in cisplatin-treated mice, found to enhance the BMC and alpha-esterase positive cells, which were drastically reduced in cisplatin alone treated control animals suggests that cisplatin-induced myelosuppression was reversed or inhibited by *X. pyrum* extract administration possibly through its chemoprotective activity. In conclusion, cisplatin and its metabolites can bind to DNA, causing damage that may result in chromosome breaks, micronucleus formation and cell death. Administration of *X. pyrum* extract in cisplatin-treated mice, found to enhance the BMC and alpha-esterase positive cells, which were drastically reduced in cisplatin alone treated control animals suggests that cisplatin-induced myelosuppression was reversed or inhibited by *X. pyrum* extract administration possibly through its chemoprotective activity.

Keywords: gastropod, *Xancus pyrum*, chemoprotective, cisplatin, alpha esterase

1. Introduction

Cancer is one of the leading causes of death around the world; it is characterized with the aid of uncontrolled growth and unfold of odd cells. If the unfold isn't managed, it can bring about demise. Cancer is resulting from both external components (tobacco, chemical compounds, radiation and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions and mutations that arise from metabolism). Those casual factors may additionally act together in series to initiate or promote carcinogenesis [1]. Malignancy is a perplexing arrangement of illnesses. Every malignancy is exceptional in the manner it develops and

builds up, its odds of spreading, the manner in which it influences one's body and the side effects one may involvement. A few components, including area and how the destructive cells show up under the magnifying instrument, decide how disease is analysed. All malignant growths, be that as it may, can be categorized as one of four general classes, for example, carcinoma, leukaemia, sarcoma and lymphoma. Carcinoma is a type of cancer developed from epithelial cells. This is the single largest group of human cancers forming about 80% of all cancers. Tumours arising from connective tissue cells (mesenchymal tissue) such as fibroblasts or bone cells are called sarcomas. Cancers of blood forming cells are called leukaemia. Sometimes cancer produced from the lymphoid origin is localized in lymph glands called lymphomas [2]. Cancer is treated with surgery, radiation, chemotherapy, hormone therapy, biological therapy and targeted therapy. Radiotherapy and chemotherapy remain the dominant weapons in the arsenal for the treatment of cancer. They kill not only the tumour cells but also normal cells [3]. An enormous number of tumours are ineffectively responsive or even non-reacting to remedial medications and radiotherapy. Expanding the dosages of cytotoxic medications and radiation neglect to improve the reaction to these treatments and a significant number of them show protection from slaughtering. A perfect methodology is distinguish anticancer specialists that trigger adequately the procedure of cell demise specially in tumour cells [4]. Radiation and chemotherapy treatments are made as they wipe out fast growing cancer cells, they can also damage fast growing normal cells. They can affect some healthy, fast growing cells causing side effects some of the most common include, Depression of the immune system, which can result in potentially fatal infections. Fatigue, the treatment can be physically exhausting for the patient, who might already be very tired from cancer-related fatigue. It may produce mild to severe anaemia. Inclination to drain effectively, prescriptions that murder quickly separating cells or platelets are probably going to lessen the quantity of platelets in the blood, which can result in wounds and dying. Amazingly low platelet checks might be incidentally helped through platelet transfusions. At times, chemotherapy medicines are delayed to permit platelet checks to recuperate. Gastrointestinal misery, nausea and regurgitating are basic symptoms of chemotherapeutic drugs that murder quick partitioning cells. This can likewise deliver loose bowels or blockage, malnutrition and lack of hydration. This can result in fast weight reduction. Male pattern baldness, a few prescriptions that slaughter quickly isolating cells cause sensational male pattern baldness; different drugs may make hair meager. These are transitory impacts: hair for the most part begins developing back half a month after the last treatment, now and again with an inclination to twist that might be known as a "chemo perm" [5].

Natural products have a long history of use in the service of mankind for the prophylaxis and treatment of several diseases and cancer is not an exception. For such a dreadful disease, apart from conventional modalities like surgery, radiotherapy and chemotherapy a few other approaches are also available or being tried. It has been reported that one-third of cancer patients use some form of complementary and other alternative medicines. In the recent times considerable attention has been focused on the identification and development of natural products for chemoprevention by systemic and rigorous screening processes many of the potential chemo preventive agents have shown considerable safety and efficacy in preclinical evaluation and are in the stages of clinical testing [6]. The class **Gastropoda** or **gastropods** (also previously known as univalves sometimes also spelled Gasteropoda) form a major part of the phylum Mollusca. Gastropods are more commonly known as snail and slugs. This is the most diversified class in the phylum with 60,000–80,000 living species. There are 409 recent families of gastropods. Fossil gastropods represent another 202 families. This class of animals is second only

to insects in its number of known species. The gastropods include many thousands of species of marine snails and sea slugs, as well as freshwater snails and fresh water limpets, and the terrestrial (land) snails and slugs [7].

The marine environment is a rich wellspring of both organic and compound biodiversity which has been investigated in the revelation of one of a kind synthetic compounds, having potential for mechanical improvement as pharmaceuticals, makeup, wholesome enhancements, atomic tests, fine synthetics and agrochemicals. As of late of novel metabolites with powerful pharmacological properties have been found from marine living being. One among them is gastropod which incorporates gastropod *Xancus pyrum* [8]. *Xancus pyrum* Linnaeus (Xancidae, Gastropoda) vernacular name Sankh shell, the moderate moving creature. The marine oils present uncommon troubles in the examination in view of the wide assortment of unsaturated fats. Though normal oils may for the most part be dissected as far as individual acids, if there should arise an occurrence of marine oils it is just conceivable to assess the different acids as per chain length. The fatty acids from the gastropod *Xancus pyrum* were obtained through extraction, isolation and chromatographic separation of visceral mass of the animals. Of the 14 fatty acids methyl esters investigated 8 were saturated fatty acids and 6 unsaturated fatty acids. Whereas out of 8 saturated fatty acids, 5 of them were the common acids. These fatty acids were used to treat cardiac diseases and obesity [9].

2. Taxonomic position of *Xancus pyrum*

2.1 Gastropod description

The word gastropod is from the Greek, gastro importance stomach and poda significance foot, thus stomach-foot, a somewhat human name dependent on the way that to people it appears that snails and slugs slither on their tummies. In actuality, snails and slugs have all their viscera, including their stomach, in a mound on the inverse, dorsal side of the body. The shell has extensive noteworthiness in Hinduism and Buddhism. It is viewed as hallowed and is one of the eight propitious images. In these religious settings the shell is now and again changed by having the tip of the tower cut off, with the goal that it tends to be blown as a formal trumpet. A few shells that are utilized along these lines are brightened with metal and semiprecious stones. The gastropod shell is the piece of the body of a gastropod or snail, a sort of mollusk. The gastropod shell is an outside skeleton or exoskeleton, which serves for muscle connection, yet additionally for security from predators and from mechanical harm. In land snails the shell is a basic insurance against the sun, and against drying out. The gastropod shell has a few layers, and is commonly made of calcium carbonate accelerated out into a natural framework known as conchiolin. The shell is discharged by a piece of the molluscan body known as the mantle. Not all gastropods have a shell, however the greater part do. In pretty much every case the shell comprises of one piece, and is ordinarily spirally snaked, albeit a few gatherings, for example, the different various families and genera of limpets, have basic cone-formed shells as grown-ups [10].

2.2 *Xancus pyrum*

Xancus pyrum, common names the chank shell, sacred chank or chank, also known as the divine conch, sometimes referred to simply as a conch, is a species of very large sea snail with a gill and an operculum. It is a marine gastropod mollusk in

the family Turbinellidae. This species occurs in the Indian Ocean. The name "chank" for the shell of this species is derived from the Indian word shankha, the divine conch. The old generic name was Turbinella. The Dutch used to call them chianco.

The shell of this species is massive, with three or four prominent columellar plicae. It is usually pure white under a heavy brown periostracum, but it can also be a pale apricot colour. It can sometimes be dotted with dark brown. The shell has impressive importance in Hinduism and Buddhism. It is viewed as hallowed and is of the eight propitious images. In these religious settings the shell is now and again altered by having the tip of the tower cut off, with the goal that it tends to be blown as a formal trumpet. A few shells that are utilized along these lines are beautified with metal and semiprecious stones. The shell of this species is quite often right - turned in its shell-looping, yet all around once in a while a left-gave, or sinistral, shell is found. In the Hindu religious setting, the exceptionally uncommon left-gave or sinistral shells of this species are known as "Dakshinavarti", instead of the more typical right-gave ones which are known as "Vamavarti". The Dakshinavarti is especially exceptionally esteemed as far as its religious importance.

Systematic position:

Kingdom: Animalia
Phylum: Mollusca
Class: Gastropoda
Clade: Neogastropoda
Superfamily: Muricoidea
Family: Turbinellidae
Subfamily: Turbinellinae
Genus: *Xancus*
Species: *X. pyrum*

3. Methodology

3.1 Animals

Innate BALB/C (6 two months) mice, gauging 23–28 g, were gotten from Pasteur Institute, rearing area, Coonoor. The creatures were housed in ventilated plastic enclosures at 37± 1°c, 40 ± 10% dampness, and 12/12-h light/dull cycles during about 14 days of acclimatization to research facility conditions and all through the whole test time frame. The creatures were encouraged with ordinary mouse chow (Sai Feeds, Mumbai, India), and given water ad libitum. Every single creature investigation was directed by the guidelines and guidelines of Animal Ethics Committee, Government of India.

3.2 Preparation and administration of extract

3.2.1 Preparation of extract

Xancus pyrum (Gastropod) were collected from Shell meat dealers, Tuticorin, South east coast of India. The Gastropod meat was washed in distilled water and dried in a hot air oven at 50°C. The dried meat was powdered and extracted overnight by stirring with 10 volumes of 75% methanol. Supernatant was collected after centrifuging at 3000 rpm for 10 min. The solvent was evaporated to dryness at 45°C in hot water bath. The yield of the extract was 10%.

3.2.2 Administration of extract

For animal administration the extract was dissolved in minimum quantity of methanol, then resuspended in 1% gum acacia in phosphate buffered saline and given at a concentration of 0.5 g/dose/animal/intraperitoneally. For in vitro experiments, the extract was dissolved in dimethyl sulfoxide (DMSO) and diluted in the medium so that the concentration of DMSO was less than 0.1%vol/vol.

3.3 Chemoprotective effect of edible gastropod meat, *Xancus pyrum*

3.3.1 Experimental protocol

The animals were divided into three groups of six animals each as follows:

Group 1: Normal animals, without any treatment.

Group 2: Treated animals received cisplatin alone dissolved in 1% gum acacia intraperitoneally for 10 consecutive days.

Group 3: Treated animals received *Xancus pyrum* (0.5 mg) methanolic extracts. Dissolved in 1% gum acacia intraperitoneally for 10 consecutive days.

3.3.2 Determination of the effects of on Xancus pyrum on lymphoid organ weight in cisplatin treated animals

Eighteen animals were randomly divided into 3 groups containing six animals each, one as normal, which did not receive any treatment. The second group as treated animals, treated with cisplatin alone. Third group treated with cisplatin and *Xancus pyrum* Three animals from each group were sacrificed at two different time intervals (7th and 11th day) by cervical dislocation. Body weight of each animal was taken before sacrifice, lymphoid organs such as thymus and spleen was excised, weighed and expressed as relative organ weight.

3.3.3 Determination of the effects of Xancus pyrum on bone marrow cellularity (BMC) in cisplatin treated animals

Bone marrow cellularity was done according to the method. Bone marrow was collected from the femur into the medium containing 2% serum and made into single cell suspension. The number of cells was determined using a haemocytometer and expressed as total cells determined by tryphan blue (1% in saline) exclusion method per femur [11].

3.3.4 Determination of the effects of Xancus pyrum on alpha esterase activity in cisplatin treated animals (azodye coupling method, Bancroft and Cook, 1984)

3.3.4.1 Principle

Esterase enzyme present or absent in monocytes hydrolyses the substrate alpha-naphthyl acetate to form an invisible primary reaction product (PRP). The complex is coupled with the diazonium salt to produce coloured final reaction product under the microscope [12].

3.3.4.2 Reagents

1. **Fixative solution.** Formaldehyde (HCHO 37%, 25 ml) acetone (45 ml), double distilled water (30 ml), disodium hydrogen phosphate (Na_2HPO_4-20 mg) and potassium dihydrogen phosphate (KH_2PO_4-100 mg).

Solution A. Pararosaline was prepared by dissolving 1 g powder in 20 ml double distilled water and 5 ml HCl. Gently warmed solution is filtered and stored in dark at 4°C.

2. **Solution B**. Sodium nitrate (4%) was prepared by dissolving 400 mg in 10 ml double distilled water.

3. **Solution C**. Alpha naphthyl acetate was prepared by 50 mg powder in 2.5 ml glycol monoethyl ether.

4. **Phosphate buffer** (pH 7.4)

5. **Harris haematoxylin.** (500 ml) was prepared by dissolving 2.5 g powder in 50 ml ethyl glycol added to a day before prepared supersaturated solution of alu (90 g in 500 ml double distilled water) and sodium iodide or potassium iodide (20 mg). The stain was stirred overnight, filtered and stored in a dark bottle.

3.3.4.3 Procedure

Bone marrow from both femurs of mice was collected in PBS, washed thrice and smeared over the slides. Air dried slides were fixed in freshly prepared fixative 30 s at 4°C and dipped in double distilled water thrice. Air dried slides were incubated at room temperature in the following freshly prepared filtered solution. 1.2 ml solution A and 1.2 ml solution B was mixed well and allowed to react for 1 min after which solution C was added and was made up to 50 ml solution by phosphate buffer (pH 7.4).

Slides were incubated in above solution for 45 min at 37°C. After incubation slides were washed in double distilled water for 10 min and counter stained with haematoxylin for 1 min. After staining slides were washed in water for long time and observed under microscope (100×, oil immersion) for scoring positive and negative alpha esterase cells out of 4000 cells.

3.4 Determination of the effect of *Xancus pyrum* on enzyme levels in cisplatin treated animals

Liver homogenates were made in ice cold Tris buffer (0.1 M pH 7.4) and was used for the estimation of SGOT, SGPT, urea and creatinine. Serum was also used to estimate all the above parameters.

3.4.1 Estimation of SGPT (Span Diagnostics Ltd., Surat, India)

3.4.1.1 Principle

Alanine aminotransferase (ALT) catalyses the transamination of L-alanine and α-ketoglutarate to form pyruvate and L-glutamate. Pyruvate so formed is coupled with 2,4-dinitrophenyl hydrazine (2,4-DNPH) to form a corresponding hydrazone, a brown coloured complex in alkaline medium and this can be measured colorimetrically [13].

$$\mathrm{L-alanine} + \alpha - \mathrm{ketoglutarate} \rightleftarrows \mathrm{pyruvate} + \mathrm{L-glutamate} \quad (1)$$

$$\mathrm{Pyruvate} + 2,4 - \mathrm{DNPH} \rightleftarrows \mathrm{corresponding\ hydrazone\ (brown\ colour)} \quad (2)$$

Reitman and Frankel method is an end-point colorimetric method for the estimation of enzyme activity.

3.4.1.2 Reagents

Reagent no.	Reagents	Composition
1.	Buffered alanine-α-KG substrate, pH 7.4	L-alanine α-KG Phosphate buffer Preservative Stabilizer
2.	2,4-DNPH Colour Reagent	2,4-Dinitrophenyl hydrazine Preservative Stabilizer
3.	Sodium hydroxide, 4 N	Sodium hydroxide
4.	Working pyruvate standard, 8 mM (150 IU/L)	Sodium pyruvate Preservative Stabilizer

Working reagent preparation:

- Reagent 1, 2 and 4 are ready to use.
- Solution I: Dilute 1 mL of Reagent 3 to 10 mL with purified water.

3.4.1.3 Procedure

Pipette into tube marked	Blank	Standard	Test	Control
Volume in mL				
Reagent 1	0.25	0.25	0.25	0.25
Serum	—	—	0.05	—
Standard	—	0.05	—	—
Mix well and incubate at 37°C for 30 min				
Reagent 2	0.25	0.25	0.25	0.25
Deionized water	0.05	—	—	—
Serum	—	—	—	0.05
Mix well and allow to stand at room temperature (15–30°C) for 20 min				
Solution I	2.5	2.5	2.5	2.5

Mix well and read the optical density against purified water in a photometer at 505 nm, within 15 min.

Calculation:

$$\text{AST (GPT) activity (IU/L)} = \frac{\text{absorbance of test} - \text{absorbance of control} \times \text{concentration of standard}}{\text{absorbance of standard} - \text{absorbance of blank}} \quad (3)$$

where concentration of standard = 150 IU/L.

3.4.2 Estimation of SGOT (Span Diagionostics Ltd., Surat, India)

3.4.2.1 Principle

Aspartate aminotranferase (AST) catalyses the transamination of L-aspartate and α-ketoglutarate to form L-glutamate and oxaloacetate. Oxaloacetate so formed is coupled with 2,4-dinitrophenyl hydrazine (2,4-DNPH) to form a corresponding hydrazone, a brown coloured complex in alkaline medium and this can be measured colorimetrically [14].

$$\mathrm{L-aspartate} + \alpha - \mathrm{ketoglutarate} \rightleftarrows \mathrm{oxaloacetate} + \mathrm{L-glutamate} \quad (4)$$

$$\mathrm{Oxaloacetate} + 2,4 - \mathrm{DNPH} \rightleftarrows \mathrm{corresponding\ hydrazone\ (brown\ colour)} \quad (5)$$

3.4.2.2 Reagents

Reagent no.	Reagents	Composition
1.	Buffered aspartate-α-KG substrate, pH 7.4	L-aspartic acid α-KG Phosphate buffer Preservative Stabilizer
2.	2,4-DNPH colour reagent	2,4-dinitrophenyl hydrazine Preservative Stabilizer
3.	Sodium hydroxide, 4 N	Sodium hydroxide
4.	Working pyruvate standard, 6 mM (114 IU/L)	Sodium pyruvate Preservative Stabilizer

Working reagent preparation:

- Reagent 1, 2 and 4 are ready to use.
- Solution I: Dilute 1 mL of Reagent 3 to 10 mL with purified water.

3.4.2.3 Procedure

Pipette into tube marked	Blank	Standard	Test	Control
Volume in mL				
Reagent 1	0.25	0.25	0.25	0.25
Serum	—	—	0.05	—
Standard	—	0.05	—	—
Mix well and incubate at 37°C for 30 min				
Reagent 2	0.25	0.25	0.25	0.25
Deionized water	0.05	—	—	—
Serum	—	—	—	0.05
Mix well and allow to stand at room temperature (15–30°C) for 20 min				
Solution I	2.5	2.5	2.5	2.5

Mix well and read the optical density against purified water in a photometer at 505 nm, within 15 min.

Calculation:

$$\text{AST (GOT) activity (IU/L)} = \frac{\text{absorbance of test} - \text{absorbance of control} \times \text{concentration of standard}}{\text{absorbance of standard} - \text{absorbance of blank}} \quad (6)$$

where concentration of standard = 114 IU/L.

3.5 Effect of *Xancus pyrum* on the biochemical parameters after cisplatin administration

3.5.1 Estimation of Urea (Span Diagnostics Ltd., Surat, India)

3.5.1.1 Principle

Urea is converted quantitatively to ammonia and CO_2 in the presence of urease. The ammonium ions react with the phenolic chromogen and hypocrite to give a green coloured complex. The intensity of the colour formed is measured at 578 nm and is directly proportional to the concentration of urea in test specimen [15].

$$\text{Urea} + H_2O \rightarrow \text{ammonia} + CO_2 \quad (7)$$

$$\text{Ammonia} + \text{phenolic chromogen} + \text{hypochlorite} \rightarrow \text{green coloured complex} \quad (8)$$

3.5.1.2 Reagents

Reagent 1: Urea (Enzyme reagent).
Reagent 2: Urea (Chromogen reagent).
Reagent 3: Urea diluents (buffer).
Reagent 4: Urea standard (50 mg/dL).

3.5.1.3 Working Reagent preparation

i. Transfer the contents of one vial of Reagent 1 to the bottle of Reagent 3.

ii. Rinse 1 Urea vial properly with 3 Urea diluents and mix gently.

iii. Store these working reagents at 2–8°C when not in use.

iv. 2 Urea reagent and Urea standard are ready to use.

3.5.1.4 Procedure

Pipette into clean and dry test tubes labelled as blank (B), standard (S) & test (T):

Addition sequence	Blank	Standard	Test
Working reagent	1000 μL	1000 μL	1000 μL
Distilled water	10 μL	—	—

Addition sequence	Blank	Standard	Test
Urea standard	—	10 μL	—
Specimen	—	—	10 μL
Mix well and incubate for 5 min at 37°C or 15 min at room temperature			
2-Urea reagent	1000 μL	1000 μL	1000 μL

1. Mix well and incubate for 5 min at 37°C or 15 min at room temperature.
2. Measure the absorbance of standard (Abs. S) & test sample (Abs. T) against the blank at 578 nm.
3. Colour is stable for 45 min when protected from light, so absorbance should be measured within that period.

3.5.1.5 Calculation

$$\text{Urea concentration in mg/dL of test specimen} = \text{Abs T} + \text{Abs S} \times 50 \quad (9)$$

$$\text{Blood urea nitrogen (BUN) in mg/dL} = \text{urea (in mg/dL)} \times 0.467 \quad (10)$$

3.5.2 Estimation of creatinine (Span Diagnostic Ltd., Surat, India)

3.5.2.1 Principle

Creatinine responds with picric corrosive in antacid medium to frame an orange hued complex. The rate of arrangement of this complex is estimated by perusing the adjustment in absorbance at 520 nm in a chose interim of time and is relative to the centralization of creatinine. The response time and the grouping of picric corrosive and sodium hydroxide have been streamlined to maintain a strategic distance from obstruction from keto acids [16].

$$\text{creatinine} + \text{picric acid} \rightarrow \text{orange coloured complex} \quad (11)$$

3.5.2.2 Reagents

Reagent 1: Picric acid.
Reagent 2: Sodium hydroxide 0.75 N
Reagent 3: Stock Creatinine Standard, 150 mg

3.5.2.3 Working solution preparation

Dilute 0.1 mL of Reagent 3 to 10 mL with purified water and mix well.

3.5.2.4 Procedure

Step A. Deproteinization of test sample

Serum/plasma	0.5 mL
Purified water	0.5 mL
Reagent 1: Picric acid	3.0 mL

Mix well, keep in a boiling water bath exactly for 1 min. Cool immediately under running tap water and centrifuge or filter.
Step B. Colour development.

	Blank (B)	Standard (S)	Test (T)
Filtrate/supernatant (from Step A)	—	—	2.0 mL
Working standard	—	0.5 mL	—
Purified water	0.5 mL	—	—
Reagent 1: Picric acid	1.5 mL	1.5 mL	—
Reagent 2: sodium hydroxide, 0.75 N	0.5 mL	0.5 mL	0.5 mL

Mix well, allow to stand at room temperature exactly for 20 min and measure immediately the optical density of blank (B), standard (S) and test (T) against purified water at 520 nm.

Calculation:

$$\text{serum creatinine in mg/100 mL} = \frac{\text{O.D.test} - \text{O.D.blank} \times 3}{\text{O.D.std.–O.D.blank}} \quad (12)$$

3.6 Statistical analysis

The results are expressed in mean ± standard deviation (SD). Statistical analysis was performed by using Students 't 'test. p values < 0.05 were considered to be statistically significant.

4. Results and discussion

4.1 Chemoprotective effect of edible gastropod meat

4.1.1 Xancus pyrum

4.1.1.1 Effect of Xancus pyrum on relative organ weights after cisplatin administration

Body weight of each animal was taken before sacrifice, lymphoid organs such as thymus and spleen was excised, weighed and expressed as relative organ weight.

The cisplatin treated animals showed high reduction in the weight of all the organs such as 0.23 ± 0.02 g/100 g body weight of spleen, 0.17 ± 0.01 g/100 g body weight of thymus, 3.7 ± 0.19 g/100 g body weight of liver, 1.2 ± 0.01 g/100 g body weight of kidney, 0.62 ± 0.01 g/100 g body weight of lungs. The cisplatin treated along with *Xancus pyrum* mice showed a significantly increase in the weight of all the organs such as 0.34 ± 0.072 g/100 g body weight of spleen, 0.23 ± 0.01 g/100 g body weight of thymus, 4.84 ± 0.05 g/100 g body weight of liver, 1.37 ± 0.18 g/100 g body weight of kidney, 0.769 ± 0.05 g/100 g body weight of lungs.

The p values of cisplatin treated animals along with *Xancus pyrum* for spleen was $p < 0.01$, which was less significant but for thymus, liver, kidney and lungs it was $p < 0.05$ which was considered to be statistically significant.

Weight of all relative organs was increased in cisplatin treated animals by the extract administration, providing supportive evidence for *Xancus pyrum* extract is immunostimulative. The results are given in **Table 1**.

Treatment	Relative organ weight (g/100 g body weight)									
	Spleen		Thymus		Liver		Kidney		Lungs	
	7th day	11th day	7th day	11th day	7th day	11th day	7th day	11th day	7th day	11th day
Normal	0.50 ± 0.11	0.69 ± 0.02	0.16 ± 0.04	0.18 ± 0.02	5.41 ± 0.38	5.98 ± 0.39	1.27 ± 0.17	1.26 ± 0.22	0.54 ± 0.03	0.59 ± 0.02
Cisplatin alone	0.23 ± 0.02	0.20 ± 0.04	0.17 ± 0.01	0.15 ± 0.07	3.78 ± 0.19	3.60 ± 0.40	1.23 ± 0.01	0.98 ± 0.04	0.62 ± 0.01	0.53 ± 0.03
Cisplatin + *Xancus pyrum*	0.34 ± 0.07**	0.41 ± 0.14**	0.23 ± 0.01**	0.26 ± 0.04*	4.84 ± 0.05*	5.58 ± 0.30*	1.37 ± 0.18*	1.43±0.08**	0.76 ± 0.05*	0.79 ± 0.08*

Values are expressed as mean ± SD.
**p < 0.05.*
***p < 0.01.*
****p < 0.001.*

Table 1.
Effect of Xancus pyrum on relative organ weights in cisplatin treated animals.

4.1.1.2 Effect of Xancus pyrum on bone marrow cellularity and α-esterase activity after cisplatin administration

Bone marrow was collected from the femur into the medium containing 2% serum and made into single cell suspension. The number of cells was determined using a haemocytometer and expressed as total cells determined by tryphan blue (1% in saline) exclusion method per femur.

Effect of *Xancus pyrum* on bone marrow cellularity and α-esterase activity is given in **Table 2**. The number of bone marrow cells as well as α-esterase positive cells was decreased drastically in cisplatin alone treated animals, but this was significantly ($p < 0.001$) reversed by administration of *Xancus pyrum*. In cisplatin treated animals, on the 7th day there was a drastic reduction in the number of bone marrow cells ($25.5 \times 10^5 \pm 1.414$ cells/femur) and α-esterase positive cells (634.5 ± 3.05 positive cells/4000 cells) compared to *Xancus pyrum* treated along with cisplatin animals. Treatment with *Xancus pyrum* could elevate the bone marrow cellularity and number of α-esterase positive cells. In cisplatin treated group of animals along with *Xancus pyrum*, bone marrow cellularity and α-esterase positive cells was found to be $68.30 \times 10^5 \pm 4.24$ cells/femur and 1179 ± 2.121 cells/4000 bone marrow cells respectively on 7th day and it was again enhanced to $69.7 \times 10^5 \pm 4.24$ cells/femur and 1227 ± 1.414 cells/4000 bone marrow cells on 11th day respectively compare to the cisplatin alone treated animals ($20.93 \times 10^5 \pm 3.055$ cells/femur and 620.66 ± 3.05 cells/4000 bone marrow cells).

4.1.1.3 Effect of Xancus pyrum on enzyme levels after cisplatin administration

4.1.1.3.1 Serum glutamic oxaloacetic transaminase (SGOT)

An enzyme that is normally present in liver and heart cells. SGPT is released into blood when the liver or heart are damaged. The blood SGPT levels are thus elevated. Also called aspartate aminotransferase (AST).

A significant increase in the levels of SGOT (82.280 ± 2.7 IU/L) and SGPT (85.22 ± 2.393 IU/L) observed in the serum samples of cisplatin alone treated group was reversed by the administration of *Xancus pyrum*. Treatment with cisplatin along with *Xancus pyrum* significantly reduced the levels of SGOT (52.68 ± 0.46 IU/L) and SGPT (55.820 ± 1.814 IU/L) in serum, that is the p value was found to be $p < 0.001$ showing that the extract is highly significant.

Treatment	**Bone marrow cellularity (cells/femur)**		**α-Esterase activity (no. of α-esterase positive cells/4000 cells)**	
Days	**7th day**	**11th day**	**7th day**	**11th day**
Normal	$85.0 \times 10^5 \pm 2.828$	$89.5 \times 105 \pm 3.536$	884 ± 2.828	892.5 ± 2.121
Cisplatin alone	$25.5 \times 10^5 \pm 1.414$	$20.9 \times 10^5 \pm 3.055$	634.5 ± 3.055	620.66 ± 3.055
Cisplatin + *Xancus pyrum*	$68.30 \times 10^5 \pm 4.242^{***}$	$69.7 \times 10^5 \pm 4.950^{***}$	$1179 \pm 2.121^{***}$	$1227 \pm 1.414^{***}$

Values are expressed as mean ± SD.
$^{*}p < 0.05$.
$^{**}p < 0.01$.
$^{***}p < 0.001$.

Table 2.
Effect of Xancus pyrum on bone marrow cellularity and α-esterase activity in cisplatin treated animals.

4.1.1.3.2 Serum glutamic pyruvic transaminase (SGPT)

An enzyme that is normally present in liver and heart cells. SGPT is released into blood when the liver or heart is damaged. The blood SGPT levels are thus elevated. Also called alanine aminotransferase (ALT).

Cisplatin treated animals showed a decrease in the levels of SGOT (32.67 ± 2.7 IU/L) and SGPT (42.04 ± 1.9 IU/L) observed in the liver sample. Administration of *Xancus pyrum* significantly increased the level of SGOT (41.545 ± 1.3 IU/L) and SGPT (48.290 ± 1.4 IU/L) in liver. The levels of SGOT and SGPT values are given in **Tables 3** and **4**. The SGPT level was increased drastically in cisplatin alone treated animals, but this was significantly ($p < 0.001$) reversed by administration of *Xancus pyrum* extract.

4.1.1.4 Effect of Xancus pyrum on the biochemical parameters after cisplatin administration

The blood urea nitrogen (BUN) test is a measure of the amount of nitrogen in the blood in the form of urea, and a measurement of renal function**. Urea** is a substance secreted by the liver, and removed from the blood by the kidneys.

The renal functions can be estimated by biochemical parameters like BUN (blood urea nitrogen) and creatinine is given in **Tables 5** and **6**. Cisplatin administration in mice was found to increase the BUN concentration in serum on 7th day 18.19 ± 0.2 mg/dL and 11th day 2.20 ± 0.04 mg/dL but this was significantly reduced to 7.00 ± 0.12 mg/dL on 7th day and 7.025 ± 0.05 mg/dL on 11th day by

Group	Serum GOT (IU/L)		Liver GOT (IU/L)	
Days	**7th day**	**11th day**	**7th day**	**11th day**
Normal	9.064 ± 0.3	9.172 ± 0.1	90.16 ± 2.9	97.41 ± 2.1
Cisplatin alone	76.92 ± 1.8	82.28 ± 2.7	43.88 ± 2.3	32.67 ± 2.7
Cisplatin + *Xancus pyrum*	55.93 ± 0.1***	52.68 ± 0.4***	39.85 ± 0.7**	41.54 ± 1.3**

Values are expressed as mean ± SD.
**p < 0.05.*
***p < 0.01.*
****p < 0.001.*

Table 3.
Effect of Xancus pyrum treatment on the serum, liver SGOT levels in cisplatin treated animals.

Group	Serum GPT (IU/L)		Liver GPT (IU/L)	
Days	**7th day**	**11th day**	**7th day**	**11th day**
Normal	9.390 ± 0.2	9.290 ± 0.04	69.45 ± 2.05	70.36 ± 0.09
Cisplatin alone	74.65 ± 1.4	85.22 ± 2.3	40.91 ± 0.96	32.04 ± 1.9
Cisplatin + *Xancus pyrum*	58.22 ± 0.3**	55.82 ± 1.8***	45.22 ± 0.9**	48.29 ± 1.4***

Values are expressed as mean ± SD.
**p < 0.05.*
***p < 0.01.*
****p < 0.001.*

Table 4.
Effect of Xancus pyrum treatment on the serum, liver SGPT levels in cisplatin treated animals.

the administration of *Xancus pyrum* extract. Similarly urea concentration in serum of cisplatin alone treated animals was increased, that is on 7th day 17.54 ± 0.4 mg/dL and on 11th day it was 19.71 ± 0.09 mg/dL which was significantly reduced to 15.01 ± 0.2 mg/dL on 7th day and 15.04 ± 0.12 mg/dL on 11th day by the administration of *Xancus pyrum* extract.

The urea level was increased drastically in serum of cisplatin alone treated animals, but this was significantly ($p < 0.001$) reversed by administration of *Xancus pyrum* extract.

Cisplatin treated animals showed an increase in the level of creatinine 1.438 ± 0.09 mg/dL on 7th day and 1.457 ± 0.08 mg/dL on 11th day in serum which was reversed to 1.07 ± 0.04 mg/dL on 7th day and 0.92 ± 0.08 mg/dL on 11th day by the administration of *Xancus pyrum* extract. It was also found that p-values was less than 0.001 ($p < 0.001$) showing that the cisplatin treated animals along with *Xancus pyrum* extract was statistically significant. Cancer is one of the dreadful diseases of this century. Radiotherapy and chemotherapy plays an important role in cancer treatment. Radiotherapy and chemotherapy is associated with toxic effect. They kill not only the tumour cell but also normal cells. Both these effects are associated with suppression of immune system. Most of the synthetic chemotherapeutic agents available today are immunosuppressant, cytotoxic and exert several side effects [17]. Modulation of immune system by cytotoxic agents is emerging as a major area in pharmacology, especially in case where undesired immunosuppression is the result of therapy. A major drawback of current cancer therapeutic practices such as chemotherapy and radiation therapy is bone marrow suppression

Group	**Serum**			
	Urea concentration (mg/dL)		**BUN concentration (mg/dL)**	
Days	**7th day**	**11th day**	**7th day**	**11th day**
Normal	28.41 ± 1.1	31.19 ± 1.4	13.26 ± 0.5	14.56 ± 0.6
Cisplatin alone	17.54 ± 0.4	4.71 ± 0.09	8.19 ± 0.20	2.20 ± 0.04
Cisplatin + *Xancus pyrum*	15.01 ± 0.2***	15.04 ± 0.12***	7.00 ± 0.12***	7.02 ± 0.05***

Values are expressed as mean ± SD.
**p < 0.05.*
***p < 0.01.*
****p < 0.001.*

Table 5.
Effect of Xancus pyrum treatment on the serum urea levels in cisplatin treated animals.

Group	**Serum (mg/dL)**	
Days	**7th day**	**11th day**
Normal	1.750 ± 0.04	1.880 ± 0.04
Cisplatin alone	1.438 ± 0.09	1.457 ± 0.08
Cisplatin + *Xancus pyrum*	1.075 ± 0.04***	0.920 ± 0.05***

Values are expressed as mean ± SD.
**p < 0.05.*
***p < 0.01.*
****p < 0.001.*

Table 6.
Effect of Xancus pyrum treatment on the serum, liver creatinine levels in cisplatin treated animals.

resulting in cytopenia and subsequent suppression of humural and cellular as well as nonspecific & specific cellular responses [18]. Weight of all relative organs was also increased in cisplatin treated animals by the extract administration, providing supportive evidence for *Xancus pyrum* extract immunostimulative potential during treatment of cisplatin. The effect of *Biophytum sensitivum* on the bone marrow cellularity and α-esterase positive cells after the administration of the methanolic extract of *Biophytum sensitivum* showed a significant ($p < 0.001$) enhancement in the bone marrow cellularity (28.3×10^6 cells/femur) compared to the normal control (17.3×10^6 cells/femur) animals. Moreover the number of α-esterase positive cells was also found to be increased significantly ($p < 0.001$) in the *Biophytum sensitivum* treated animals (1421 cells/4000 bone marrow cells) compared to the normal animals (905 cells/4000 bone marrow cells [19]. Similarly, the effect of *Xancus pyrum* on the bone marrow cellularity and α-esterase positive cells after the administration of the methanolic extract of *Xancus pyrum* showed a significant ($p < 0.001$) enhancement in the bone marrow cellularity in cisplatin treated animals, there was a drastic reduction in the number of bone marrow cells ($25.5 \times 10^5 \pm 1.414$ cells/femur) and α-esterase positive cells (634.5 ± 3.05 positive cells/4000 cells) compared to *Xancus pyrum* treated along with cisplatin animals. Treatment with *Xancus pyrum* could elevate the bone marrow cellularity and number of α-esterase positive cells. In cisplatin treated group of animals along with *Xancus pyrum*, bone marrow cellularity and α-esterase positive cells was found to be $69.7 \times 10^5 \pm 4.24$ cells/femur and 1227 ± 1.414 cells/4000 bone marrow cells. In the present study, chemoprotective effect of *Xancus pyrum* an important edible gastropod was found in mice. Administration of *Xancus pyrum* was found to increase the number of bone marrow cells significantly indicating the extract could stimulate the haematopoetic system. Moreover there was increased presence of α-esterase positive bone marrow cells indicating the extract treatment could also enhance the differentiation of stem cells. The extract was found to stimulate the weight of spleen and thymus indicating that *Xancus pyrum* stimulated the production of immune cells. The increased SGOT and SGPT levels in the serum of cisplatin treated mice can be attributed to the damaged structural integrity of the liver and kidney, because these enzymes are located in cytoplasm and are released into circulation after cellular damage [20]. The present study showed that *Xancus pyrum* extract had decreased the SGOT and SGPT levels in the serum during the cisplatin treatment in mice. The blood urea nitrogen (BUN) test is a measure of the amount of nitrogen in the blood in the form of urea, and a measurement of renal function. Urea is a substance secreted by the liver, and removed from the blood by the kidneys. The most common cause of an elevated BUN is poor kidney function. A greatly elevated BUN (>60 mg/dL) generally indicates a moderate-to-severe degree of renal failure. Impaired renal excretion of urea may be due to temporary conditions such as dehydration or shock, or may be due to either acute or chronic disease of the kidneys themselves [21]. Cisplatin administration in mice was found to increase the BUN concentration in serum and liver on 7th day and 11th day but this was significantly reduced to by the administration of *Xancus pyrum* extract, thus it's clear that the poor kidney function was enhanced by the *Xancus pyrum* extract. Cyclophosphamide treated animals showed an increase in the level of creatinine 1.536 ± 0.0603 mg/dL on 11th day and 1.526 ± 0.03 mg/dL on 15th day in serum which was reversed to 1.17 0.08 mg/dL on 11th day 0.87 mg/dL on 15th day by the administration of *Bauhinia tomentosa* [22].

Similarly, cisplatin administration in mice was found to increase the level of creatinine 1.438 ± 0.09 mg/dL on 7th day and 1.457 ± 0.08 mg/dL on 11th day in serum which was reversed to 1.07 ± 0.04 mg/dL on 7th day and 0.92 ± 0.08 mg/dL on 11th day by the administration of *Xancus pyrum* extract. Many well-recognized problems are associated with excessive intake of dietary fat, including obesity,

insulin resistance, coronary heart disease, and some forms of cancer. While intakes of saturated, trans, and arachidonic fatty acids have been linked to the development of chronic disease, research shows omega-3 (n-3) fatty acids, specifically fish oils or in marine mollusks are essential in the prevention and treatment of disease. It is scientifically proven marine gastropod—*Xancus pyrum* is an edible gastropod which contains 8–10% of protein, 4–5% of carbohydrates 2–3% of minerals, and 1–2% of fat. This also contains omega 3 fatty acids [23]. The cisplatin treated animals showed high reduction in the weight of all the organs such as 0.23 ± 0.02 g/100 g body weight of spleen, 0.17 ± 0.01 g/100 g body weight of thymus, 3.7 ± 0.19 g/100 g body weight of liver, 1.2 ± 0.01 g/100 g body weight of kidney, 0.62 ± 0.01 g/100 g body weight of lungs. The cisplatin treated along with *Xancus pyrum* mice showed a significantly increase in the weight of all the organs such as 0.34 ± 0.072 g/100 g body weight of spleen, 0.23 ± 0.01 g/100 g body weight of thymus, 4.84 ± 0.05 g/100 g body weight of liver, 1.37 ± 0.18 g/100 g body weight of kidney, 0.769 ± 0.05 g/100 g body weight of lungs.

The p-values of cisplatin treated animals along with *Xancus pyrum* for spleen was $p < 0.01$, which was less significant but for thymus, liver, kidney and lungs it was $p < 0.05$ which was considered to be statistically significant. The number of bone marrow cells as well as α-esterase positive cells was decreased drastically in cisplatin alone treated animals, but this was significantly ($p < 0.001$) reversed by administration of *Xancus pyrum*. In cisplatin treated animals, on the 7th day there was a drastic reduction in the number of bone marrow cells ($25.5 \times 10^5 \pm 1.414$ cells/femur) and α-esterase positive cells (634.5 ± 3.05 positive cells/4000 cells) compared to *Xancus pyrum* treated along with cisplatin animals. Treatment with *Xancus pyrum* could elevate the bone marrow cellularity and number of α-esterase positive cells. In cisplatin treated group of animals along with *Xancus pyrum*, bone marrow cellularity and α-esterase positive cells was found to be $68.30 \times 10^5 \pm 4.24$ cells/femur and 1179 ± 2.121 cells/4000 bone marrow cells respectively on 7th day and it was again enhanced to $69.7 \times 10^5 \pm 4.24$ cells/femur and 1227 ± 1.414 cells/4000 bone marrow cells on 11th day respectively compare to the cisplatin alone treated animals ($20.93 \times 10^5 \pm 3.055$ cells/femur and 620.66 ± 3.05 cells/4000 bone marrow cells).

A significant increase of the levels of SGOT (82.280 ± 2.7 IU/L) and SGPT (85.22 ± 2.393 IU/L) observed in the serum samples of cisplatin alone treated group was reversed by the administration of *Xancus pyrum*. Treatment with cisplatin along with *Xancus pyrum* significantly reduced the levels of SGOT (52.68 ± 0.46 IU/L) and SGPT (55.820 ± 1.814 IU/L) in serum. Cisplatin treated animals showed a decrease in the levels of SGOT (32.67 ± 2.7 IU/L) & SGPT (42.04 ± 1.9 IU/L) observed in the liver sample. Administration of *Xancus pyrum* significantly increased the level of SGOT (41.545 ± 1.3 IU/L) and SGPT (48.290 ± 1.4 IU/L) in liver. It was also found that p-values was less than 0.001 ($p < 0.001$) showing that the cisplatin treated animals along with *Xancus pyrum* extract was statistically significant.

The renal functions can be estimated by biochemical parameters like BUN (blood urea nitrogen) and creatinine. Cisplatin administration in mice was found to increase the BUN concentration in serum on 7th day 18.19 ± 0.2 mg/dL and 11th day 2.20 ± 0.04 mg/dL but this was significantly reduced to 7.00 ± 0.12 mg/dL on 7th day and 7.025 ± 0.05 mg/dL on 11th day by the administration of *Xancus pyrum* extract. Similarly urea concentration in serum of cisplatin alone treated animals was increased, that is on 7th day 17.54 ± 0.4 mg/dL and on 11th day it was 19.71 ± 0.09 mg/dL which was significantly reduced to 15.01 ± 0.2 mg/dL on 7th day and 15.04 ± 0.12 mg/dL on 11th day by the administration of *Xancus pyrum* extract. It was also found that p-values was less than 0.001 ($p < 0.001$) showing that the cisplatin treated animals along with *Xancus pyrum* extract was statistically

significant. Cisplatin treated animals showed an increase in the level of creatinine 1.438 ± 0.09 mg/dL on 7th day and 1.457 ± 0.08 mg/dL on 11th day in serum which was reversed to 1.07 ± 0.04 mg/dL on 7th day and 0.92 ± 0.08 mg/dL on 11th day by the administration of *Xancus pyrum* extract. It was also found that p-values was less than 0.05 ($p < 0.05$) showing that the cisplatin treated animals along with *Xancus pyrum* extract was statistically significant.

In the past several decades, thousands of marine compounds with tremendous pharmacological activities have been isolated and more than a dozen of them are in different stages of human clinical trials against various diseases. Thus from above mentioned experiment it is clearly known that the gastropod *Xancus pyrum* has reduced the side effects that is been caused by cisplatin (chemo drug). In the present study, chemoprotective effect of *Xancus pyrum* an important edible gastropod was studied. Administration of *Xancus pyrum* was found to increase the number of bone marrow cells significantly indicating the extract could stimulate the haematopoetic system. Moreover there was increased presence of α-esterase positive bone marrow cells indicating the extract treatment could also enhance the differentiation of stem cells. The extract was found to stimulate the weight of spleen and thymus indicating that *Xancus pyrum* stimulated the production of immune cells.

5. Conclusion

Cisplatin and its metabolites can bind to DNA, causing damage that may result in chromosome breaks, micronucleus formation and cell death. Administration of *Xancus pyrum* extract in cisplatin treated mice, found to enhance the bone marrow cellularity and α-esterase positive cells, which were drastically reduced in cisplatin alone treated control animals suggests that cisplatin induced myelosuppression was reversed or inhibited by *Xancus pyrum* extract administration possibly through its chemoprotective activity.

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