

RESEARCH ARTICLE

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Extraction and Characterization of Chick PEA (*Cicer arietinum*) Extract with Immunostimulant Activity in BALB/C MICE

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Abstract

Traditional plant medicines are used for a range of cancer conditions. The chickpea is highly proteinaceous and consumed as a staple in many parts of the world. An evaluation of chemoprotective and immunomodulatory activities of *Cicer arietinum* (CE) in cisplatin-induced immunosuppressed mice was here performed. Cisplatin was given at 100mg/kg, intraperitoneally, and after induction of immunosuppression mice were treated with *Cicer arietinum* (0.5 mg/dose/animal/IP) for a period of 10-day. The influence of the extract on lymphoid organ weight, bone marrow cellularity, alpha esterase activity and on enzyme levels such as (SGOT,SGPT, Urea, Creatinine) was assessed to identify any chemoprotective influence. Administration of CE to cisplatin-treated mice alleviated the drastic reduction in bone marrow cellularity and α - esterase positive cells seen with cisplatin. Thus myelosuppression was reversed or inhibited. Cisplatin binds to DNA and causes damage resulting in chromosome breaks, micronucleus formation and cell death. CE is comprised of numerous middle-chain aliphatic alcohols, aldehydes and ketones, in addition to compounds like 7-hydroxy-1-methoxy-6-methylanthraquinone, cyclohexadecane (CAS) and 6-(amino methyl)-2-naphthol. These latter are thought to contribute to the characteristic aroma of *C. arietinum*. In conclusion, administration of CE in cisplatin-treated mice, boosted bone marrow cellularity and increased α - esterase positive cells, thus reversing myelosuppression.

Keywords: *Cicer arietinum*- chemoprotective- cisplatin- alpha esterase- histopathology

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Introduction

Cancer is caused by several factors (UV, radiation, tobacco, mutation, abnormal cell division) that includes both external and internal. Carcinogenesis is initiated by these mutations occurred (Proksch et al., 2002). A WHO Survey conducted in 2015-2017 states clearly that human cancers are forming about 80 percent of all cancers (Mayer et al., 2003). Treatment involves radiation, chemotherapy, hormone therapy, biological therapy and targeted therapy (Garcia et al., 2002). The best cure could be relying on identifying anticancer agents that stops the rapid multiplication of cells leading to cell death (Haefner,2003). This can also produce diarrhea or constipation, Malnutrition and dehydration. This can result in rapid weight loss. Hair loss (Goodman et al., 2002). The chickpea (*Cicer arietinum*) is an edible legume of the family Fabaceae, subfamily Faboideae. Chickpeas are high in protein and one of the earliest cultivated vegetables; 7,500-year-old remains have been found in the Middle East. The plant grows to between 20 and 50 cm high and has small feathery leaves on either side of the stem (Girdhani et al., 2005). Chickpeas are grown in the Mediterranean, western Asia, the Indian subcontinent and

Australia. The ancient Romans used many different types of herbs and plants for medicinal purposes. Chickpeas are mentioned in Charlemagne's Capitulare de villis (about 800 CE) as *cicer italicum*, as grown in each imperial demesne. Glandular secretion of the leaves, stems, and pods consists of malic and oxalic acids, giving a sour taste. In India these acids used to be harvested by spreading thin muslin over the crop during the night. In the morning the soaked cloth is wrung out, and the acids are collected in bottles (Jaiprakash et al., 2000; Bouchet, 1991). Chick Peas has got several medical applications like treatment of ulcer, bronchitis, cholera, constipation thus these magical seeds are considered as antibilious (Pillay et al., 1959).

Material and Methods

Identification and Purification of Bioactive Compounds
Phytochemical Analysis of Cicer arietinum Extract by High Performance Thin Layer Chromatography (HPTLC)

The phytoconstituents present in the organic extracts were determined qualitatively by HPTLC. In HPTLC, the extracts spotted on silica coated plates, were developed using butanol-glacial acetic-water (100: 10:10) as the solvent system. The HPTLC results were further used to

validate the presence of alkaloids based on positive reaction (brown coloration) with Dra-gendorff's reagent, steroids based on positive reactions (violet to blue or green) with acetic anhydride and H₂SO₄ and flavonoids with 1% Ethanolic Aluminium chloride reagent followed by terpenoids using Anisaldehyde sulphuric acid reagent. The bioactive and phytoconstituents like alkaloid, flavonoid, steroid, terpenoids was determined using (Keller-Killani test) (Nammi et al.,2003).

GC-MS Analysis of Bioactive Components in Methanolic Extract of Cicer arietinum

Gas Chromatography-mass spectrometry(GC-MS) is used in identification of several compounds including volatile, non-volatile and thermally stable compounds. Dried extract of CE was dissolved in 95% v/v methanol and analyzed using GC Clarus 500, PerkinElmer, USA and equipped with Turbo mass gold-pek-in Elmer Detector and split injection system. 2µl of sample was injected for analysis and the analysis was carried out. The unknown component was compared to NIST library (Madhuri et al., 2009).

Animals

Inbred BALB/C (6-8 weeks) mice, weighing 20- 25 g, were obtained from Pasteur Institute, breeding section, Coonoor. The animals were housed in ventilated Plastic cages at 37 ±1 °C, 40±10% humidity (Richard et al., 2008). All animal experiments were conducted according to the rules and regulations of Animal Ethics Committee, Government of India.

Preparation and administration of Extract

Fresh seeds of Cicer arietinum was bought from Arunachal Pradesh, India. The seeds were dried at 50 °C (Annapurna et al., 2017). The dried seeds was powdered and extracted overnight by stirring 75% methanol. Supernatant was collected after centrifuging at 3,000 rpm (Niladri et al., 2007). Rotary evaporator was used to remove the solvent in invacuo (Raphael et al., 2003). The yield of the extract was 10%. 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 gm / 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 (Uttamdatta et al.,2003).

Experimental Design

The animals were divided into three groups of six animals each as follows: (Sallie et al.,1991)

Group 1: Normal animals, without any treatment.

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

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

Determination of the influence of C. arietinum on lymphoid organ weight in Cisplatin treated animals

Eighteen animals were randomly separated containing six animals each, one as normal, which stayed without any treatment. The second group as treated animals that includes cisplatin only. Third extract (CE) with Cisplatin. Three animals from each group were sacrificed at two different time intervals (7th and 11th day) by cervical dislocation. Weight of each animal was analyzed (Devasagayam et al.,2002).

Effect of C. arietinum on bone marrow cellularity (BMC) in Cisplatin treated animals

Sredni et al., (1992) procedure was used to analyse the BMC. 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 tryphan blue (1% in saline) exclusion method. Haemocytometer was used to analyse the total number of cells expressed (Alagarwami et al., 1965).

Effect of C. arietinum on alpha esterase activity in Cisplatin treated animals

This study was carried out as per Azodye coupling method (Bancroft and Cook, 1984) with little modification. Esterase enzyme present 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 colored final reaction product under the microscope (Appukuttan, 1976). Femurs was separated to PBS, washed thrice and smeared over the slides. Slides were fixed after air drying in freshly prepared fixative 30 sec at 4 °C and dipped in double distilled water thrice. After that the fixed slides were incubated in freshly prepared filtered solution. 1.2 ml solution A and 1.2 ml solution B was mixed well and allowed after which solution C was added and was made up to 50 ml solution by Phosphate buffer (pH 7.4). After hematoxylin staining slides were washed in water for long time and viewed using microscope (100x, oil immersion) for scoring positive and negative alpha esterase cells out of 4,000 cells.

Effect of C. arietinum 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.

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

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 colored complex in alkaline medium and this can be measured calorimetrically (Ping et al.,2003). Aspartate aminotransferase (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 calorimetrically (Ferri, 1998).

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

Urea is converted quantitatively to ammonia and CO₂ 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. Creatinine reacts with picric acid in alkaline medium to form an orange coloured complex (Toro et al., 1975).

Statistical Analysis

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

Results

Phytochemical Analysis of *Cicer arietinum* Extract by HPTLC

HPTLC of CE was carried to identify the phytoconstituents (alkaloid, steroid, flavonoids and terpenoids) availability Figure 1 and Table 1.

GC-MS Analysis of Bioactive Components in Methanolic Extract of *C. arietinum*

Cicer arietinum were found to contain numerous middle-chain aliphatic alcohols, aldehydes and ketones, which are considered to be the end products of fatty acids. Besides compounds like 7-Hydroxy-1-methoxy-6-methylanthraquinone, Cyclohexadecane (CAS and 6-(Aminomethyl)-2-naphthol were also identified Figure 2. This identified compounds contribute to the characteristic aroma. Table 2.

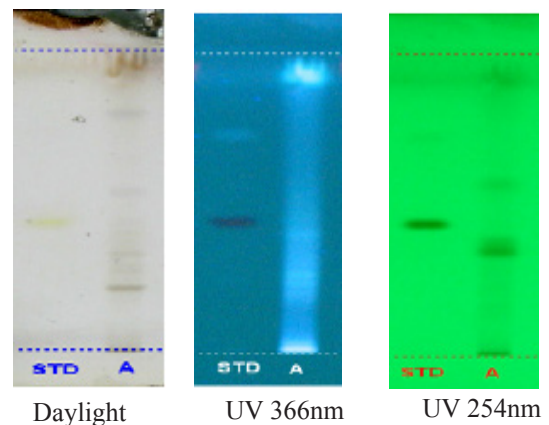
Effect of *C. arietinum* on Relative organ weights after Cisplatin administration.

Weight of animals was identified before sacrificing them. After sacrifice the organs like lymphoid, kidney, spleen was excised and weighed accordingly Table 3. The Cisplatin treated animals showed high reduction in the weight of all the organs such as 0.23 \pm 0.02 g/100 g body weight of spleen, 0.17 \pm 0.01 g/100 g body weight of thymus, 3.7 \pm 0.19 g/100 g body weight of liver, 1.2 \pm 0.01 g/100g body weight of

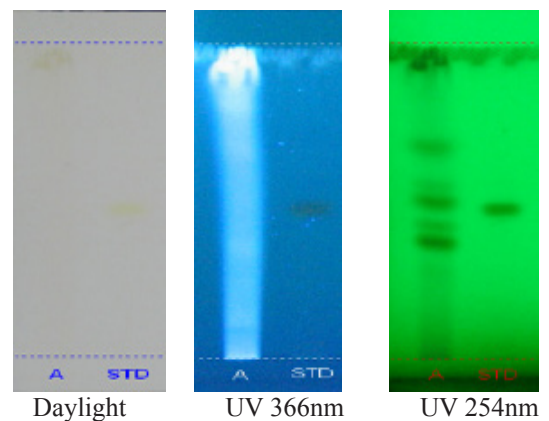
Table 1. Preliminary Phytochemical Screening of *C. arietinum* Extract

Test for Extract	Inference
Alkaloid	+
Flavanoid	+
Steroid	+
Terpenoid	+

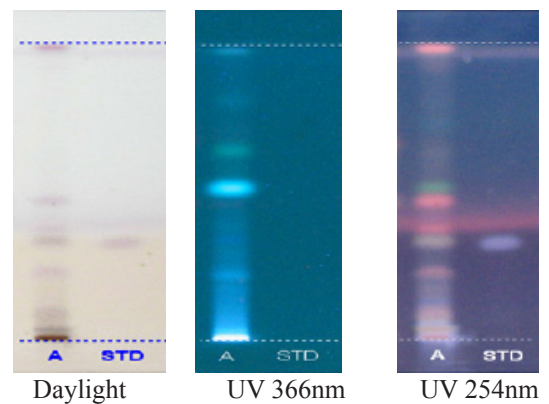
+, Presence; -, Absence



TLC Plates Showing the Presence of Alkaloid



TLC Plates Showing the Presence of Flavanoid



TLC plates showing the presence of Steroid

Figure 1. Phytochemical Standardization of *Cicer arietinum* by HPTLC

kidney, 0.62 \pm 0.01 g / 100 g body weight of lungs. The Cisplatin treated along with extract, the mice showed a significantly increase in the weight of all the organs such as 0.34 \pm 0.072 g / 100 g body weight of spleen, 0.23 \pm 0.01g/100 g body weight of thymus, 4.84 \pm 0.05 g /100 g body weight of liver, 1.37 \pm 0.18 g / 100 g body weight of kidney, 0.769 \pm 0.05 g /100 g body weight of lungs. The p Values of Cisplatin treated animals with extract for spleen was p<0.01, which was less significant but for thymus, liver, kidney and lungs it was p<0.05 which were significant Weight of all relative organs was increased in cisplatin due to CE extract administration, providing supportive evidence for extract is immune stimulative.

Effect of C. arietinum on Bone Marrow Cellularity and α-esterase activity after Cisplatin administration

Effect of extract on bone marrow cellularity and α-esterase activity is given in Table 4. 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 extract. In Cisplatin treated animals, on the 7th day there was a decrease ($25.5 \times 10^5 \pm 1.414$ cells / femur) and α-esterase positive cells (634.5 ± 3.05 positive cells / 4,000 cells) related to CE treated along with cisplatin animals. Treatment with the above extract could elevate the bone marrow cellularity and number of α-esterase positive cells. In Cisplatin treated group of animals along with chick pea, 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).

Effect of C. arietinum on enzyme levels after Cisplatin administration

Serum Glutamic Pyruvic Transaminase (SGOT) also called aspartate aminotransferase (AST) (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 the extract Table 5, Table 6. Treatment with Cisplatin along with the extract 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 high significant. The SGPT is also known as alanine amino transferase (ALT). Cisplatin treated animals showed a decrease in the levels of SGPT (32.67 ± 2.7 IU/L) and SGPT (42.04 ± 1.9 IU/L). Administration of extract increases SGPT (41.545 ± 1.3 IU/L) and SGPT (48.290 ± 1.4 IU/L) in liver Table 5 and Table 6. The SGPT level was increased drastically in cisplatin only treated animals, but this was significantly ($p < 0.001$) reversed by CE extract.

Effect of C. arietinum on the biochemical parameters after Cisplatin administration

The renal functions can be estimated by biochemical

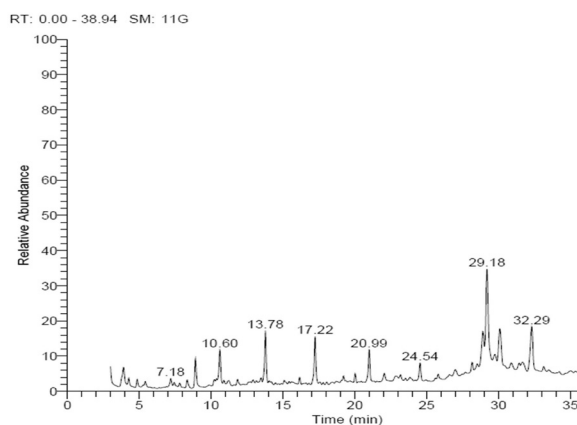


Figure 2. GC-MS analysis of *Cicer arietinum*

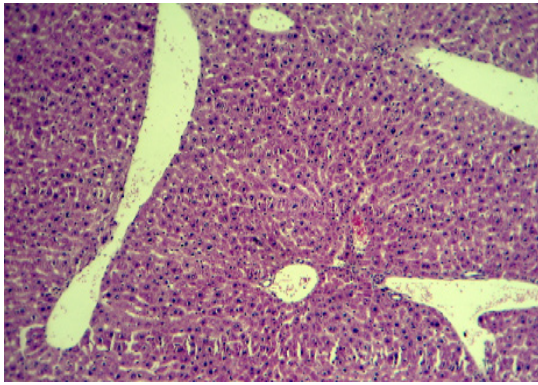
parameters like BUN (Blood Urea Nitrogen) and Creatinine is given in the Table 7 and Table 8. Cisplatin administration in mice increases the BUN concentration in serum on 7th day 18.19 ± 0.2 mg/dL and 11th day 2.20 ± 0.04 mg/dL but decreased to 7.00 ± 0.12 mg/dL on 7th day and 7.025 ± 0.05 mg/dL on 11th day by CE extract. Similarly, Urea concentration in serum of cisplatin only 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 extract. The Urea level was increased drastically in serum of Cisplatin alone treated animals, but this was significantly ($p < 0.001$) reversed by CE extract. Cisplatin treated animals had increased 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. P- Values was less than 0.001 ($p < 0.001$) showing that the Cisplatin treated animals along with CE extract was statistically significant.

Histopathological Examination of Liver

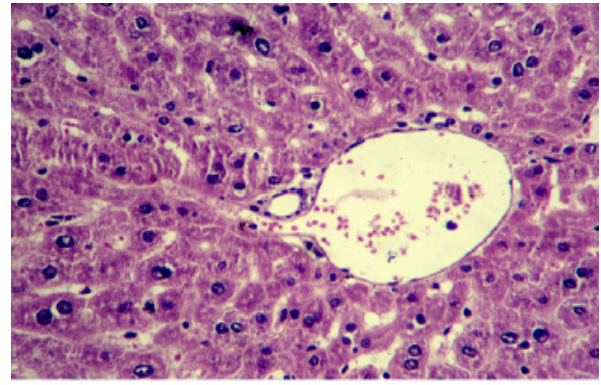
Histopathological examination of liver of cisplatin induced immunosuppressed mice and control had remarkable changes. These showed infiltration with focal necrosis of hepatocytes Figure 4. The normal healthy control liver tissue sections showed liver parenchyma with hepatocytes which appear normal Figure 3 and .Immunosuppressed of *C. arietinum* extract Figure 5. revealed a remarkable improvement of hepatic tissues

Table. 2 Bioactive Components Identified in Methanolic Extract of *C. arietinum* by GC-MS Analysis

S.No	Retention time (RT)	Rf Value	Name of the compound	Molecular formula	Molecular weight
1	29.18	6.00,3	7-Hydroxy-1-methoxy-6-methylanthraquinone	C ₁₆ H ₁₂ O ₄	268
2	32.29	6.00,3	6-(Aminomethyl)-2-naphthol	C ₁₁ H ₁₁ NO	173
3	13.78	6.00,3	Cyclohexadecane (CAS)	C ₁₆ H ₃₂	224
4	17.22	6.00,3	(cis)-2-nonadecene	C ₁₉ H ₃₈	266
5	20.99	6.00,3	1-formylbenzo[b]fluoranthene	C ₂₁ H ₁₂ O	280
6	10.60	6.00,3	1-Tetradecene (CAS)	C ₁₄ H ₂₈	196
7	24.54	6.00,3	(cis)-2-nonadecene	C ₁₉ H ₃₈	266
8	7.18	6.00,3	3-Dodecene, (Z)-	C ₁₂ H ₂₄	168

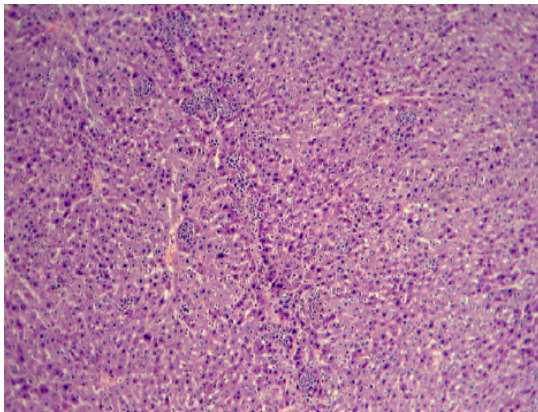


A) Normal Lobular Architecture

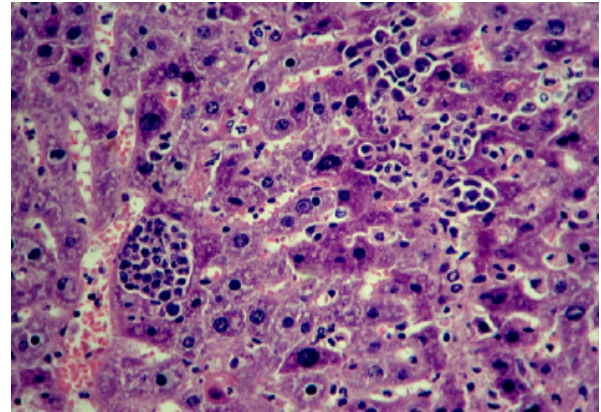


B) Normal Central Vein and Sinusoids

Figure 3. Histopathological Changes in Liver of Normal Healthy Control Mice

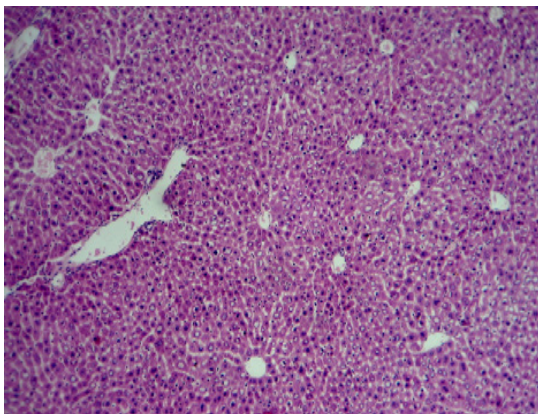


A) Lobular Inflammation

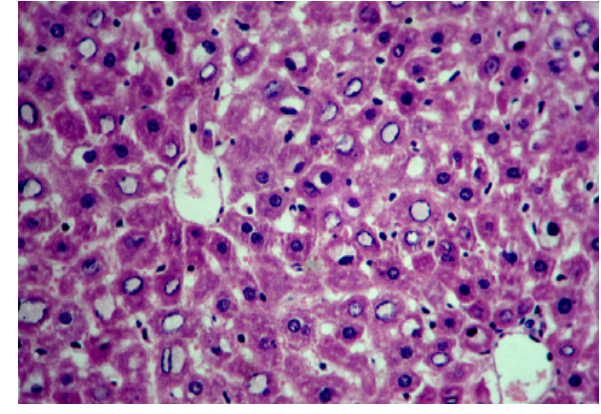


B) Central Vein Congestion and Sinusoids with Inflammatory Cells

Figure 4. Histopathological Changes in Liver of Cisplatin Induced Immunosuppressed Mice



A) Normal Lobular Structure with no Inflammation



B) Normal Architecture in Central Vein and Sinusoids

Figure 5. Histopathological Changes in Liver of *Cicer Arietinum* Treated MiceTable 3. Effect of *C.arietinum* on Relative Organ Weights in Cisplatin Treated Animals

Treatment	Relative organ weight (g/100g body weight)									
	Spleen		Thymus		Liver		Kidney		Lungs	
	7 th day	11 th day	7 th day	11 th day	7 th day	11 th day	7 th day	11 th day	7 th day	11 th 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 +Cicer arietinum	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 4. Effect of *C.arietinum* on Bone Marrow Cellularity and α-esterase Activity in Cisplatin Treated Animals

Treatment	Bone Marrow Cellularity (Cells/femur)		α-Esterase activity (No. of α- esterase positive cells/4000 cells)	
	7 th day	11 th day	7 th day	11 th day
Normal	85.0×10 ⁵ ±2.828	89.5×10 ⁵ ±3.536	884± 2.828	892.5± 2.121
Cisplatin alone	25.5 × 10 ⁵ ± 1.414	20.9×10 ⁵ ± 3.055	634.5 ± 3.055	620.66 ± 3.055
Cisplatin + Cicer arietinum	68.30×10 ⁵ ± 4.242***	69.7×10 ⁵ ± 4.950***	1179± 2.121***	1227± 1.414***

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

Table 5. Effect of *C.arietinum* Treatment on the Serum, Liver SGOT Levels in Cisplatin Treated Animals

Group	Serum GOT (IU/L)		Liver GOT (IU/L)	
	7 th day	11 th day	7 th day	11 th 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 + Cicer arietinum	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 6. Effect of *C.arietinum* Treatment on the Serum, Liver SGPT Levels in Cisplatin Treated Animals

Group	Serum GPT (IU/L)		Liver GPT (IU/L)	
	7 th day	11 th day	7 th day	11 th 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 + Cicer arietinum	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 7. Effect of Cicer Arietinum Treatment on the Serum Urea Levels in Cisplatin Treated Animals

Group	Serum(mg/dL) Urea concentration (mg/dL)		BUN concentration (mg/dL)	
	7 th day	11 th day	7 th day	11 th 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 + Cicer arietinum	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 8. Effect of *C.arietinum* Treatment on the Serum, Liver Creatinine Levels in Cisplatin Treated Animals

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

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

where reduced necrosis was observed and found to be normal in CE treated mice.

Discussions

Cancer is a dreadful disease of this century. Treatment involving radiations and chemo drugs kill not only the tumor cell even the healthy cells. Both these effects are associated with suppression of immune system (Ayoola et al., 2008). Bone marrow damages suppresses the humoral and specific cellular responses

(Sredni et al., 1992).

Weight of all relative organs amplified in cisplatin animals by the CE extract administration, providing supportive evidence for extract. 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 x 10⁶ cells/femur) compared to the normal control (17.3 x 10⁶ cells /femur) animals. Moreover, the number of a-esterase positive cells was also found to be increased significantly (p<0.001) in the Biophytum sensitivum treated animals (1,421 cells/4,000 bone marrow cells) compared to the normal animals (905 cells/ 4,000 bone marrow cells (Guruvayoorappan et al., 2007).

Similarly, the effect of *C.arietinum* on the bone marrow cellularity and α- esterase positive cells after the administration of the methanolic extract 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 / 4,000 cells) compared to extract treated along with cisplatin animals. Treatment with the extract could elevate the bone marrow cellularity and number of α - esterase positive cells. In Cisplatin treated group of animals along with extract the 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 / 4,000 bone marrow cells.

In the present study, chemo shielding property of *C. arietinum* an important edible chick pea was found in mice. CE increases the bone marrow cells significantly indicating the extract could stimulate the hematopoietic system. Moreover, there was increased presence of α -esterase activity proving that the extract treatment could also enhance the differentiation of stem cells The extract was found to stimulate the weight of spleen and thymus indicating the stimulated production of immune cells. The increased SGOT and SGPT levels in the serum of Cisplatin treated mice can be ascribed to the damaged structural integrity of the liver and kidney, because all this enzyme is located in cytoplasm and are released into circulation after cellular damage (Gordon et al., 2009). The present research identified and proved that the extract had decreased the SGOT and SGPT levels in the serum during the cisplatin treatment in mice. A greatly elevated BUN (>60 mg/dL) generally indicates a moderate-to-severe degree of renal failure. Impaired renal excretion of urea the temporary conditions such as dehydration or shock, or may be due to either acute or chronic disease of the kidneys themselves (Barnard et al., 1974).

Cisplatin administration in mice increases the BUN concentration in serum and liver on 7th day and 11th day but this decreased to by CE extract, thus it's clear that the poor kidney function was enhanced by the *C. arietinum* extract. Cyclophosphamide treated animals had increased 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 1.17 ± 0.87 mg/dL on 15th day by *Bauhinia tomentosa* (Badami et al., 2003). Similarly, Cisplatin administration in mice increased the 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 7 th day and 0.92 ± 0.08 mg/dL on 11th day by CE extract. It is scientifically proven *C. arietinum* 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 (Greenwald, 1991; Brown et al., 1985; Raghavan et al., 2006). Treatment with extract brought back the cellular arrangement to near normal. Neither erythrocyte hemorrhage nor inflammatory cell infiltration were encountered. Similarly, the effect of *T. arjuna* stem bark extract on histopathology of liver cisplatin-induced toxicity (Ikawati et al., 2006).

In conclusion, cancer is curable if treated from the beginning. The potential role of the CE as dietary anti-oxidants is the main mechanism for their preventive nature against cancer and inflammatory diseases. The main agenda was to access the immunomodulatory activity of chick pea in cisplatin induced immunosuppressed mice

and to identify the influence on relative organ weight, bone marrow cellularity, α -esterase activity, enzyme levels and biochemical parameters. Cisplatin binds to DNA, causing damages finally leading to cell death. Administration of extract in cisplatin treated mice increases bone marrow cellularity and α - esterase positive cells, which were drastically reduced in immunosuppressed suggests that cisplatin induced myelosuppression was reversed or inhibited by *C. arietinum* extract administration by its chemo protective activity

Declaration of Interest

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