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Original Articles

Role of Vitamin C and Spirulina on Cisplatin Induced Interstitial Nephritis in Long Evans Rats

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Abstract:

Context: *Cisplatin, an effective antineoplastic agent, is the drug of choice in the treatment of germ cell tumours. It is one of the important nephrotoxic drugs. Oxidative stress is suggested to be a significant contributor to cisplatin-induced nephrotoxicity. So, vitamin C and spirulina having antioxidant properties are expected to deal with the situation. The study was designed to observe the effects of vitamin C and spirulina on cisplatin-induced interstitial nephritis in Long Evans rats.*

Study design: *Experimental*

Place and period of study: *Department of Anatomy, Sir Salimullah Medical College, Dhaka, from September, 2005 to December, 2006.*

Materials and Methods: *Forty adult Long Evans rats, of either sex, weighing 220-300 gms each, were divided into four equal batches depending on the drug treatment they received: (a) 'normal control' – distilled water and normal saline, (b) 'experimental control' – cisplatin, (c) 'vitamin C treated' – cisplatin plus vitamin C, and (d)'spirulina treated' – cisplatin plus spirulina. Histologically, nephrotoxicity was determined by the presence of inflammatory changes in the interstitium.*

Results: *Nephrotoxic control rats showed marked interstitial nephritis as evidenced by histological features. Pretreatment with either vitamin C or spirulina reduced the cisplatin-induced interstitial nephritis ($p<0.001$), while spirulina was more effective than vitamin C as evidenced histologically ($P< 0.001$).*

Conclusion: *It can be concluded from this study that both spirulina and vitamin C have preventive impact on cisplatin-induced interstitial nephritis in rats.*

Key words: *Cisplatin, interstitial nephritis, vitamin C, spirulina*

Introduction:

Cancer possesses a serious health problem both in developed and developing countries. In Bangladesh, cancer has become a matter of serious concern with increase of the incidence of this disease. According to the Bangladesh Cancer Society there are around 800,000 cancer patients in the country of the 130 million people and about 200,000 new cases are being added every year. Around 150,000 cancer patients die annually.[1] At present about 50% of patients with cancer can be cured with combined therapy.[2]

Cisplatin, a Platinum co-ordination complex, is the drug of choice in the treatment of germ cell tumours.[3]

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But its use is restricted due to its dose limiting nephrotoxicity[4]. Though the kidneys constitute less than 1% of total body mass, they receive approximately 20% of the cardiac output. As the kidneys concentrate and excrete metabolic waste, chemicals and many drugs, it is often exposed to toxic concentration of these substances greater than other tissues. The acid pH of the fluid within most of the nephron segments can also influence the local concentration and solubility of the toxins thus making the kidneys vulnerable to toxic injuries. As oxidative stress is thought to be a significant contributor to cisplatin-induced nephrotoxicity, it has been suggested that, use of antioxidants can counteract it [5]. So, vitamin C and spirulina having antioxidant properties are expected to deal with the situation.

Materials and methods:

An experimental study was carried out in the Department of Anatomy, Sir Salimullah Medical College from September, 2005 to December, 2006 on forty adult healthy Long Evans rats of either sex weighing 220 to 300 gms each and aged between 10 to 12 weeks. The rats were allocated into 4 batches containing 10 rats in each batch depending on their treatment schedule. Grouping of the rats and their treatment are shown in Table -I.

Drugs: The following drugs were used in the study:

1) Inj.Cisplatin: A single dose of 6 mg/kg body weight was given intraperitoneally slowly [6,7]
2) Spirulina powder: a dose of 1000mg/kg body weight/day. Spirulina suspension was made in distilled water and administered through intragastric feeding[8].
3) Vitamin C: Inj. Vitamin C was given at a dose of 50mg/kg body weight subcutaneously into the anterior abdominall wall[9].
4) Vehicle: Both normal saline and distilled water were used for normal control batch.

Grouping of the animals and their treatment regimens are shown in Table –II.

For preparing blocks for histological sections, each kidney was halved through the hilum to obtain a maximum area for light microscopy. One half of each kidney was considered to be the representative of the entire organ. Then a paraffin block was prepared from each half-kidney. Thus, one block represented one rat and a good section made from each block also represented one rat. Thus, 6 good slides from each group were selected randomly for histological study. Each histologic section of the kidney was divided into 10 subdivisions. For this purpose, a figure with 10 subdivisions was computer generated and was photographically reproduced on a transparent plastic sheet in an appropriate size Fig.-1. This sheet was then fixed over the cover slip on the slide[10]. Microscopically kidney is composed of uriniferous tubules, blood vessels, and interstitial tissue. Interstitium is situated in between the tubules and blood vessels. Estimation of percentage volume of interstitial tissue was done by using a 'point-counting' technique[11] by applying stereology. Stereology is the three-dimensional interpretation of flat images (sections and projections) by the criteria of geometric probability. It is practiced by measuring and / or counting profiles in sections and is done by inserting an engraved pattern into the eye-piece. In the 'point-counting' technique, the volume of a component is determined by the number of points (profile) that hit that component, divided by the total number of test points, which equals the volume fraction of that component in the entire specimen.

To measure the percentage volume of the interstitial tissue in the kidneys by'point-counting' technique, a replica of Zeiss type-1 integrating eye-piece was computer generated and was photographically reproduced on a transparent plastic sheet in an appropriate size. The plastic sheet was placed into the eye-piece of the microscope. The integrating eye- piece contains a network of 25 points as shown in Fig.-2.

The counting was done under light microscope in high magnification, using an X 40 objective (Fig.-3). In this magnification, each subdivision of the kidney section was focused and the position of each point hitting the interstitium was duly recorded. 5 fields were chosen from each subdivision starting from the centre of the section of the kidney towards the cortex randomly,

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because, percentage of interstitial tissue is more in the medulla than the cortex. So, a total number of 50 fields were counted in each slide. Thus taking 25 points of the eye-piece for each field a total of (25x50) or 1250 point-positions were recorded for each section (i.e.every rat). The total number of points hitting the interstitium was summed up and expressed as a percentage of the total number of points hitting the renal microstructures. This percentage represented the proportional volume of interstitial tissue in each kidney section (i.e.each rat).

Results:

The percentage volume of interstitial tissue in different batches of rats are presented in Fig.-4. It is noted from fig.-4, that the normal control rats had minimum amount of interstitial tissue, whereas it was maximum in the experimental control rats. There was significant increase in the percentage volume of interstitial tissue in experimental control rats in comparison to normal control rats ($P<0.001$). The percentage volume of interstitial tissue was reduced both in vitamin C treated and spirulina treated rats in comparison to experimental control rats and both the values were statistically significant($P<0.001$). The percentage volume of interstitial tissue was higher in the vitamin C-treated rats than that of the spirulina treated rats. Statistically the difference between the two batches was highly significant ($P<0.001$).

Table-I

Grouping of the rats into different batches

Name of the batch	Number of rats(n)	Drug or alternate
Normal control (Batch- A)	10	Normal saline + Distilled water
Experimental control (Batch- B)	10	Cisplatin
Vitamin C- treated (Batch- C)	10	Cisplatin + Vitamin-C
Spirulina- treated (Batch- D)	10	Cisplatin + Spirulina

Table-II

Experimental design showing grouping of the rats and their treatment

Batch	Name and dose of the drugs	Administration of the drugs	Duration of treatment	Day of sacrifice
Normal control (n=10) (Batch-A)	Distilled water 2 ml/rat + Normal saline 1.5 ml/rat	Oral + intraperitoneal	Day 1- Day 8 On Day 4	Day 11
Experimental control (n=10) (Batch-B)	Cisplat in 6 mg/kg body wt.	intraperitoneal	On Day 4	Day 11
Vitamin C-treated (n=10)(Batch-C)	Vitamin – C 50 mg/kg body wt.+Cisplatin 6 mg/kg body wt..	Subcutaneous + intraperitoneal	Day 1 – Day 8 On Day 4	Day 11
Spirulina-treated (n=10)(Batch-D)	Spirulina 250 mg/rat + Cisplatin 6 mg/kg body wt.	oral + intraperitoneal	Day 1- Day 8 On Day 4	Day 11

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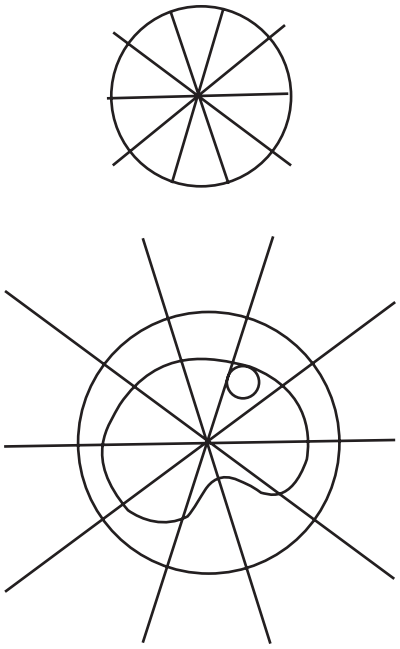


Fig.-1: *Process of dividing the kidney tissue on histological slides for examining the interstitial*

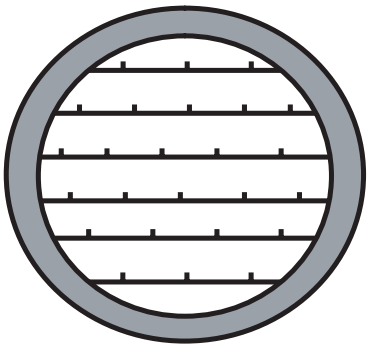


Fig.-2: *Figure of an integrating eye-piece with 25 points which was used to measure the interstitial tissue of the kidney*

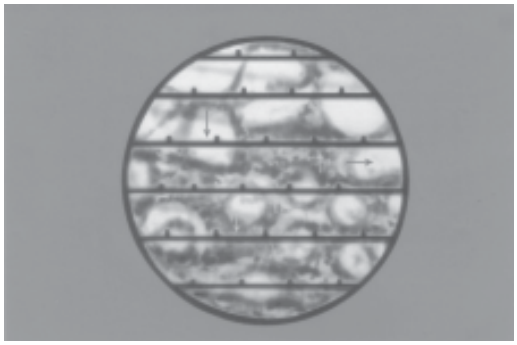
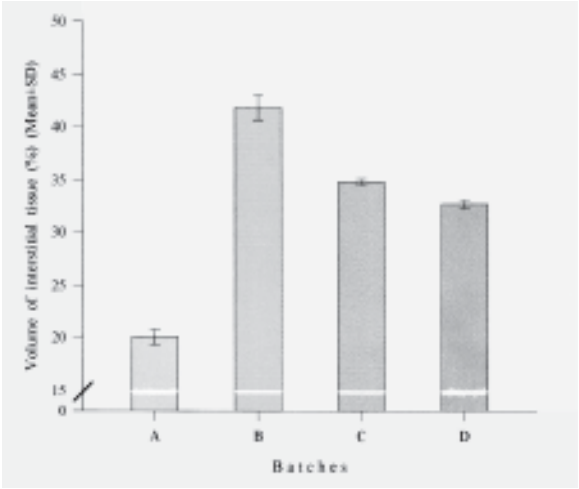


Fig.-3: *Photomicrograph of a kidney while looking through an integrating eyepiece, showing interstitial tissue (yellow arrow) and tubules (blue arrow).*



Batch A : Normal control
Batch B : Experimental control
Batch C : Vitamin C treated
Batch D : Spirulina treated

Fig. 4: *Percentage volume of interstitial tissue of different batches of rats (n=6 in each batch)*

Discussion:

The percentage volume of interstitial tissue in the spirulina-treated batch was lower than that of the experimental control rats, which was highly significant (P<0.001). The study of Mao et al., 2005[12], supported the present study, who observed the anti-inflammatory action of spirulina in the reduction of allergic rhinitis suggestive of improvement in the inflammatory condition with spirulina treatment.

Comparisons between the observations and results in the vitamin C-treated rats and those in the spirulina-treated rats could provide an idea about which dietary antioxidant is better in the prevention of cisplatin-induced interstitial nephritis. When the results of both the batches were compared with each other, better results were observed in the spirulina-treated rats than that of the vitamin C-treated rats and the differences were highly significant(P<0.001). These were supported by the study of Appenroth et al.(1997)[13], who observed better histological features in the rats received vitamin E than that of the rats received vitamin C along with a single dose of cisplatin.

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Conclusion:

After reviewing and comparing the findings of the present study in different batches of rats, it may be concluded that the level of nephrotoxicity produced by cisplatin was reduced by either vitamin C or spirulina. However, neither vitamin C nor spirulina proved to be able to keep the nephrotoxic status of the rats close to normal level. Although from the trends, spirulina was observed to be more effective in this prevention than vitamin C and spirulina-treated rats showed better histological features while compared with those of the vitamin C treated rats. Lastly, spirulina is a high protein containing micro-algae, containing about 60-70% of its dry weight. With such a high protein content it exerts protective effects against cisplatin-induced nephrotoxicy. As protein is being restricted in the treatment of any form of acute renal failure, if this protein fraction of spirulina is removed, it would become more effective against cisplatin-induced nephrotoxicity.

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