CHEMO-PROTECTIVE EFFECT OF TURMERIC AND SPIRULINA ON CISPLATIN INDUCED TOXICITY IN REPRODUCTIVE SYSTEM OF MALE ALBINO RAT, *Rattus norvegicus*

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**ABSTRACT**

Although Cisplatin is an effective chemotherapeutic drug, but generation of reactive oxygen species and mitochondrial dysfunction has limited its usefulness due to its toxicity to normal cells including testis cells. This research was designed to investigate the toxicity of chemotherapeutic drug Cisplatin and possible chemo-protective effect of Turmeric and Spirulina on Cisplatin induced toxicity on reproductive system of male albino rat. In the result, it is found that, Cisplatin targets rapidly dividing germ cells and it therefore results in the impairment of spermatogenesis leading to the depletion in sperm motility, sperm count and alteration in biochemical and testicular histology. Turmeric and Spirulina shows chemo-protective effect in rats treated with Cisplatin, as amelioration is found in all the parameters. However, Spirulina shows more chemo-protection than Turmeric. It is known that, antioxidants are present in Turmeric and Spirulina (Curcumin in Turmeric and C-phycocyanin and β-carotene in Spirulina) which acts as a free radical scavenger, is might be the reason behind ameliorative role. Altogether, after the findings of this study, beneficial effects of using a free radical scavenger such as Turmeric and Spirulina are suggested, to minimize the Cisplatin-associated testicular toxicity.

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INTRODUCTION
Chemotherapy involves the use of chemical agents to stop the growth and eliminate cancer cells even at distant sites from the origin of primary tumor. However, it does not differentiate between a cancer and normal cells and eliminates not only fast growing cancer cells in the body but also other fast growing normal cells in the body.

Cisplatin (Cis-diammine dichloro platinum), is an important anticancer drug used to treat solid tumors. Although it is very effective in suppressing cancer cells in various organs or tissues [1,2], this drug has various side effects [3,4]. Cisplatin inhibits nucleic acid synthesis is apparently responsible for the anti-tumour action and the regression of the seminiferous epithelium, it may also affect on spermatogenesis through its action upon Leydig cells testosterone production [5] or Sertoli cells [6].

Reactive oxygen species (ROS) is a recently recognized mechanism in the pathogenesis of testicular toxicity induced by Cisplatin in experimental studies [7,8]. Cisplatin causes Lipid peroxidation (LPO) and degrades the activity of enzymes that protect testicular tissue from oxidative damage in Cisplatin treated rats [9]. Various studies have shown that exposure to Cisplatin disrupts the redox balance of tissues, directly act on cell components, including lipids, proteins and DNA suggesting that biochemical and physiological disturbances result from oxidative stress [10,11]. Several studies have reported that numerous signaling pathways are involved in modulating cell survival or apoptosis in response DNA damage induced by Cisplatin [12], and these signaling pathways can also be activated by oxidative stress and lipid peroxidation [13,14].

Turmeric (Curcuma longa) is widely used as a spice, colouring material and food preservative in India, China and South East Asia. Turmeric (Tur) contains carbohydrates (69.4%), moisture (13.1%), protein (6.3%), fat (5.1%), and minerals (3.5%). The essential oil (5.8%) obtained by steam distillation of rhizomes has α-phellandrene (1%), 1,8-cineole (1%), borneol (0.5%), zingerberene (25%) and sesquiterpenes (53%) [15]. Curcumin (diferuloylmethane) (3–4%) is responsible for the yellow colour, and comprises curcumin (94%), curcumin II (6%) and curcumin III (0.3%) [16]. Curcumin has been known to be a potential anti-inflammatory agent [17,18] and antioxidant [19,20] with bioprotective and phytonutrient properties.

Spirulina (Arthospira platensis), a blue-green algae, is rich in proteins, carotenoids and other micronutrients [21]. The composition of the Spirulina (Spi), used in our experiments, was made up of proteins (65.38%), crude phycocyanin (15.37%), minerals (7.95%), total carotenoids (4.3 mg/g), β-carotene (1.67 mg/g) and total pheophorbide (0.02%) [22]. The extracts of Spirulina possess antioxidant [23], anticancer [24], antiviral [25], hepatoprotective [26], immune enhancing [27] and lipid-lowering [28] effects. Spirulina is known to possess significant antioxidant and free-radical scavenging properties [29]. C-phycocyanin effectively inhibits carbon tetrachloride-induced lipid peroxidation in rat liver and prevents hepatotoxicity [30]. Protective effect of Spirulina has been found against Doxorubicin induced cardiotoxicity [31]. The present investigation was designed to find out the toxicological effects of Cisplatin on reproductive system of male albino rats, furthermore two dietary supplements Turmeric and Spirulina were used to study their possible protective and ameliorative properties against toxicity caused by Cisplatin.

MATERIALS AND METHODS

Test Chemical
Cisplatin was obtained from Cipla Pharmaceuticals. Fresh Turmeric rhizomes were purchased and fine powder of it was formed in Laboratory of Endocrinology, Department of Bioscience, Barkatullah University, M.P. Spirulina Capsules manufactured by Cosmic Neutra Cons Solutions Pvt., Limited, H.P. were purchased.

Animals and Treatment
Healthy mature male albino rats having weight of 125±5 gm were used for the experiments. Animals were kept at ambient room temperature of 26 ± 2°C with a relative humidity of 75%, under a controlled 12 hour light/dark cycle. The rats were reared on standard diet and tap water ad libitum. The use of animal was as per Control and Supervision of Experiments on Animals (CPCSEA) norms. Animals were acclimatized to their environment for one week prior to experimentation. Animals were housed in the Animal House of the Laboratory of Endocrinology, Bioscience Department, Barkatullah University, and Bhopal.

Twenty experimental animals were divided into four groups of five each.

- **Group 1:** The animals of this group fed with balanced diet and water ad libitum and served as control. This group received intra-peritoneal injection of normal saline 0.9% (3ml/kg bw) on 1st, 4th and 7th day.
- **Group 2:** The animals of this group fed with balanced diet and water ad libitum and received three dose of intra-peritoneal injection of Cisplatin (3mg/kg bw) on 1st, 4th and 7th day.
Group 3: The animals of this group fed with balanced diet, water *ad libitum* and received three dose of intra-peritoneal injection of Cisplatin (3mg/kg bw) on 1\textsuperscript{st}, 4\textsuperscript{th} and 7\textsuperscript{th} day and supplemented with Turmeric powder (300mg/kg bw) daily, orally with the help of feeding canulla till sacrifice.

Group 4: The animals of this group fed with balanced diet, water *ad libitum* and received three dose of intra-peritoneal injection of Cisplatin (3mg/kg bw) on 1\textsuperscript{st}, 4\textsuperscript{th} and 7\textsuperscript{th} day and supplemented with Spirulina powder (1000mg/kg bw) daily, orally with the help of feeding canulla till sacrifice.

On the 10\textsuperscript{th} day of the treatment all the animals of control and experimental groups were sacrificed by cervical dislocation.

**Statistical Analysis**

All the data of experimental findings were calculated by using statistical tools and results were expressed as mean and SEM. Students t-test were performed and the differences were considered significant when \( p < 0.05 \).

**Sperm Analysis**

The epididymal sperm were collected by cutting epididymis of cauda region into small pieces and flushing the sperm in normal saline. The epididymal fluid was subjected to sperm motility and sperm count using Neubauer haemocytometer.

**Biochemical Estimation**

After every rat was sacrificed by cervical dislocation, testes were quickly dissected out in 0.9% of normal saline washed thoroughly, cleared from adherent tissue debris and the blotted dry. The tissues were then used to prepare homogenates and to estimate total amount of the tissue protein, fructose, alkaline phosphatase (ALP) and acid phosphatase (ACP) level. Fructose estimation was done by modified method of Foreman *et al.* [33]. Protein estimation was done by Follin-Phenol method of Lowry *et al.*[34], whereas ACP and ALP estimated by method of Bergmeyer and King [35].

**Histological Studies**

The testes were collected from all the groups, fixed in Bouin’s fluid, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and mounted in molten paraffin wax. Fine micron sections of 5µm were obtained, stained with Hematoxylin and Eosin and evaluated for any structural changes under student’s microscope as done by Ehrlich [36].

**OBSERVATION & RESULTS**

**Sperm Analysis**

We can see that rats treated with Cisplatin only, shows total decrease in their sperm motility i.e. 1.5%, as compared to the control, i.e. 74%, in Fig. (1). Though the sperm motility was not normal in Cisplatin + Turmeric (33.1%) and Cisplatin + Spirulina (48.2%), but sperm motility is significantly increased as compared to the Cisplatin Only treated rats. The average sperm count in control rats is 74.12±0.46 millions/ml. As shown in Fig. (2), a significant decreased has been observed in rats treated with Cisplatin Only, 35.12±0.14 millions/ml. However in groups, in which Cisplatin was supplemented with Turmeric and Spirulina, sperm count 42.27±0.32 millions/ml and 46.17±0.27 millions/ml was observed, respectively.

**Enzyme Estimation**

There are remarkable changes in phosphatase enzyme level of rats treated with Cisplatin Only. Acid phosphatase enzyme activity in Cisplatin Only treated rats showed significant increase. Alkaline phosphatase enzyme activity showed significant decrease in the same group. ACP (Fig.3) and ALP (Fig.4) enzyme activity in groups treated with Cisplatin and supplemented with Turmeric and Spirulina shows less alteration as compared with Cisplatin only group. There is no variation on testicular Protein content (Fig.5). Significant increase in Fructose level is observed in groups treated with Cisplatin only, (Fig.6).

**Table 1:** Effect of Cisplatin Only, Cisplatin + Turmeric and Cisplatin + Spirulina on different parameters of Control and treated rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Cis Only</th>
<th>Cis+Tur</th>
<th>Cis+Spi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm Motility (%)</td>
<td>74 ± 0.24</td>
<td>1.5 ± 0.06***</td>
<td>33.1 ± 0.16***</td>
<td>48.2 ± 0.27***</td>
</tr>
<tr>
<td>Sperm Count millions/ml</td>
<td>74.12 ± 0.46</td>
<td>35.12 ± 0.14***</td>
<td>42.27 ± 0.32***</td>
<td>46.17 ± 0.27***</td>
</tr>
<tr>
<td>ACP (IU/gm tissue)</td>
<td>5.20 ± 1.12</td>
<td>23.4 ± 1.01***</td>
<td>17.49 ± 1.43***</td>
<td>13.23 ± 1.66***</td>
</tr>
<tr>
<td>ALP (IU/gm tissue)</td>
<td>2.18 ± 0.07</td>
<td>0.93 ± 0.49***</td>
<td>1.29 ± 0.07**</td>
<td>1.94 ± 0.09</td>
</tr>
<tr>
<td>Protein Content(mg/gm tissue)</td>
<td>115.2 ± 0.2</td>
<td>117.3 ± 0.4</td>
<td>118.0 ± 0.6</td>
<td>118.0 ± 0.4</td>
</tr>
<tr>
<td>Fructose Content(µg/mg tissue)</td>
<td>0.32 ± 0.01</td>
<td>0.52 ± 0.013***</td>
<td>0.45± 0.016***</td>
<td>0.42 ± 0.015***</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM. * = p < 0.05, ** = p < 0.01 & *** = P < 0.001.
Figures 1: Sperm Motility

Figures 2: Sperm Count

Figures 3: Acid Phosphatase Activity

Figures 4: Alkaline Phosphatase Activity

Figures 5: Protein Content

Figures 6: Fructose Content in Control

Cisplatin only (Cis only), Cisplatin+Turmeric (Cis+Tur) and Cisplatin+Spirulina (Cis+Spi) groups. Results are expressed as mean ± SEM. NS = Non Significant, * = p < 0.05, ** = p < 0.01 & *** = P < 0.001.
Histology
The testicular histo-architectural observations Fig. 7(B) of rats treated with Cisplatin only showed degenerative changes. Sections show completely empty seminiferous tubules. Interstitial cells of Leydig were not well distributed and their size was diminished as compared to the Fig. 7(A) control batch. Testicular morphology showed less degeneration in rats supplemented with Turmeric with Cisplatin Fig. 7(C). Seminiferous tubules and interstitial cells of Leydig were less affected as compared to the Cisplatin only batch. However, batch supplemented with Spirulina Fig. 7(D) showed testicular morphology near to control batch.

![Figure 7: Histological examination of rat testis.](image)

**A** Regular seminiferous tubules with normal germinal epithelium in control rats. The connective tissue stroma was well distributed. Interstitial cells of Leydig were also well distributed and showed normal shape and size.

**B** Irregular seminiferous tubules with severe depletion of spermatogenesis in rats treated with Cisplatin only. C) Some empty seminiferous tubules and interstitial cells of Leydig were not well distributed; their size was less affected in Cisplatin + Turmeric treated rats. D) Regular seminiferous tubules showing spermatogenesis at level of spermatocytes in Cisplatin + Spirulina treated rats.

**DISCUSSION**
A large number of researches is going on in the field of oxidants and antioxidants and had attracted widespread interest in nutrition research, biology and medicine. It has become clear that constant generation of pro-oxidants, including oxygen free radicals, is an essential attribute of aerobic life [37]. An imbalance in the pro-oxidant/antioxidant system has been defined as oxidative stress. Reactive oxygen species (ROS) are very reactive molecules known as free radicals because of presence of unpaired electron such as a superoxide ion ($\mathrm{O}_2^-$), nitrogen oxide (NO) and hydroxyl radical (HO$^\cdot$). Oxidative damage to biomolecules and hyperactivation of ROS signaling pathways influence regulation of cell growth and transformation processes.[38] Although it is naturally present in the organism, they are mainly confined to cell compartments and counter balanced by natural antioxidant molecules, such as catalase, lipid peroxidase, superoxide dismutase, glutathione peroxidase, glutathione, vitamin E and vitamin C, acting as free radical scavengers [39]. Although a number of studies have demonstrated some side effects of the chemotherapeutic drug Cisplatin on male reproductive system, the present study is the first comprehensive study revealing the comparative chemoprotective effect of Turmeric and Spirulina on male reproductive system at enzymatic and cellular levels.

In the current study, Cisplatin leads to the decrease in Sperm motility and Sperm Count. Significant increase in testicular ACP level and fructose content was reported whereas significant decrease in ALP level was found in rats treated with Cisplatin only. The
Amelioration is observed in spermatogenesis among Turmeric and Spirulina delivered rats may be associated with the antioxidant and free radical scavenger properties of them.

Apart from damaging DNA, Cisplatin induces testicular damage by causing oxidative stress with the formation of ROS that can trigger cell death. In the present research, testicular damage induced by Cisplatin treatment was characterized by decrease in sperm count, sperm motility, significant alteration in enzyme level and spermatogenesis compared with untreated control animals. It has been reported that the male rats receiving Cisplatin show a decrease in reproductive organ weights and impaired fertility along with alterations on the growth and development of the next generations [40,41]. In the current study, it was determined that the administration of Cisplatin significantly decreased the epididymal sperm count, sperm motility, altered biochemical and histopathological architecture due to marked parenchymal atrophy. Histopathological examination showed severe degeneration, necrosis and reductions in seminiferous tubules alongside reduction in germinal cell thickness in the testes of rats treated with Cisplatin Only. However, in the present study, Turmeric and Spirulina treatment shows chemoprevention in all the parameters in the Cisplatin+Turmeric and Cisplatin + Spirulina group of rats when compared with those in the Cisplatin only group. This protective effect of Turmeric and Spirulina can be explained by the fact that antioxidant i.e. Curcumin and C–phytocyanin present respectively prevents cellular damage occurring as a result of oxidative stress in spermatogenic cells of seminiferous tubules and Leydig cells of the stroma.

CONCLUSION
Our results show that treatment with Turmeric and Spirulina confers cytoprotection against the deleterious effects caused by Cisplatin preventing cellular death. Antioxidants (Curcumin in Turmeric and C–phytocyanin and β-carotene in Spirulina) present in them acts as a free radical scavenger and improve testicular condition. Considering the damage to spermatogenesis caused by Cisplatin, there is a potential risk of impaired seminal quality and harm to reproductive capacity in patients treated with Cisplatin. Together, the findings of this study, we suggest beneficial effects of using a free radical scavenger in protecting Cisplatin-associated testicular toxicity. Hence, it is expected that Turmeric and Spirulina may find application as a novel drug in the near future to cure various diseases including carcinogenesis and oxidative stress induced pathogenesis. It is also suggested that extensive research on its bioactivity, mechanism of action, pharmacotherapeutics and toxicology may provide novel therapeutic insight related to oncogenic research.

AUTHOR'S STATEMENT
The authors declare no conflict of interest.

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