MODULATING EFFECTS OF CURCUMA LONGA (TURMERIC) EXTRACT AGAINST ADRIAMYCIN INDUCED GENOTOXICITY IN SWISS ALBINO MICE

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ABSTRACT

In the present investigation, studies were carried out to observe the modulating effects of crude Curcuma longa rhizome extract (CRE) against Adriamycin (ADR) induced genotoxicity in germ cells of mice using Sperm Morphology Assay. Two experiments were conducted in germ cells of Swiss albino male mice. Three different doses 150, 250 and 350mg/kg body weight of the Curcuma longa extract and 16mg/kg body weight Adriamycin were taken for study based on therapeutic concentration., in first test the antigenotoxic effects of Curcuma longa were studied and in the second test one group of animals were treated with Adriamycin alone and other groups of animals were primed with Curcuma longa extract prior to adriamycin treatment. Control animals were fed only with physiological saline. All the exposed and control Animals were sacrificed on last day of fifth week and cauda epididymis was dissected out, smear was made and stained with Eosin, slides were prepared and screened for the presence sperm abnormalities. The results were found to be insignificant, where Curcuma longa extract proved to be antigenotoxic in treated animals A significant increase in the percentage of abnormal sperms were noted when treated with Adriamycin alone and the cells showed inhibition in the percentage of abnormal sperms when animals were primed with Curcuma longa extract prior to adriamycin treatment. The present study reveals that the Curcuma longa extract was found to be antimutagenic. Hence Cucuma longa supplementation is safer in chemotherapeutic strategy.

KEYWORDS: Curcuma longa, genotoxicity, Adriamycin, germcells, Swiss albino mice.
INTRODUCTION
Adriamycin is an anti-cancer chemotherapy drug and derived by chemical semisynthesis from a bacterial species. Adriamycin works by intercalating DNA. It is commonly used in the treatment of a wide range of cancers. It is frequently used in combination chemotherapy as a component of various chemotherapy regimens. In its unaltered form, adriamycin has presented great treatment potential, being regarded as one of the most potent of the Food and Drug Administration-approved chemotherapeutic drugs.\(^1\) The ability of adriamycin to fight against rapidly dividing cells and slow down the disease progression has been widely recognized for several decades, limited only by its toxicity on non-cancerous cells in the human body. An antineoplastic drug, adriamycin was tested for its mutagenic potential in \textit{in vivo} mouse system using analysis of chromosomal aberrations in germ cells of mice. A significant increase in the frequency of chromosomal aberrations in germ cells of mice was observed at all dose levels. The results conclude that Adriamycin is capable of inducing clastogenic activity in mouse system.\(^2\)

Alternative treatments founded in a back to nature approach might yield improved treatment avenues with fewer or no undesirable side effects. In the search of this new treatment, natural products are carving a path as prospective anticancer agents. Antioxidants act as antimutagens and defenses against DNA damages caused by various agents. Antioxidants which act as antimutagens are able to protect non-malignant (normal) cells and organs against damage caused by cytostatic agents.\(^3\) Natural antioxidants in the human diet can attenuate the effects of mutagens and genotoxic carcinogens. An increase in the dietary content of antioxidants through the increased ingestion of fruits, vegetables and spices like turmeric, black pepper, garlic, ginger etc, rich in these compounds can decrease the oxidation of DNA by free radicals, thereby preventing cancer and other degenerative diseases.

Natural Products are attaining additional focus because of their less toxicity and high efficacy against a number of disorders. Epidemiological studies have revealed that fruits, vegetables, spices, tea and medicinal herbs rich in antioxidants and other micronutrients protect against diverse forms of chemically induced carcinogenesis, inhibit DNA-damage, mutagenesis and lipid peroxidation.\(^4\) It is important to reduce toxicity in normal cells, a goal that can be achieved by concurrent administration of free radical scavenging agents, such as antioxidants.\(^5,6\) Equalizing the effects of mutagenic agents are the antimutagenic/anticarcinogenic action of a diverse range of chemicals found in a diversity of foods. It is
well known that consumption of fruits and vegetables is related and are known to prevent chromosomal and DNA damage in animals.\cite{7} Usually antimutagens acting in rodents are active in human too.\cite{8}

*Curcuma longa* has been used for broad spectrum of diseases and its isolated compound curcumin found to be anticarcinogenic and a potential antioxidant. In the present investigation studies were carried out to observe the efficacy of crude *Curcuma longa* extract against adriamycin induced genotoxicity in germ cells of mice. Turmeric is an ancient spice derived from the rhizomes of *Curcuma longa*, which is a member of the Zingiberaceae family is a perennial herb. Also known as ‘Golden Spice of India’ turmeric has been used in India for medicinal purposes for centuries. Its rhizomes and oils have great importance. It is extensively used as spice in domestic cooking. In combination with other natural dyes, it is also used as a coloring agent for textiles, pharmaceuticals, confectionary and cosmetics.\cite{9} Asian medicine for generations for the treatment of many disorders, inflammation of skin, wounds, hepatic and biliary disorders, cough as well as certain tumours.\cite{10} It has shown to have a wide spectrum of biological actions; these include anticarcinogenic, antimutagenic and antibacterial properties.\cite{11,12,13} Curcumin an active ingredient of *Curcuma longa* showed antioxidant and anti-inflamatory effects.\cite{14} and cardioprotective nature.\cite{15} Hence in the present investigation an effort has been made to test the efficiency of *Curcuma longa* against adriamycin induced genotoxic damage in germ cells of mice using Sperm Morphology Assay.

**MATERIALS AND METHODS**

**Procurement and Identification of Plant Material**

The plant material that is fresh rhizomes of *Curcuma longa* were procured from wholesale spice and herbs market, Hyderabad and were identified in Department of Botany, Osmania University, Hyderabad. Fresh *Curcuma longa* rhizomes were cleaned and washed with deionised water, sliced and dried in the sun for one week and again dried at 50°C in a hot air oven for 6 hours. Dried rhizomes were cut in small pieces, powdered by electronic mill.

**Drugs and Chemicals**

Adriamycin of Pfizer company was bought from Apollo Pharmacy, Hyderabad and Eosin stain (Himedia). The chemicals and glassware used in the study are purchased from Rahul Scientifics, Hyderabad, Telangana.
Preparation of the extract

*Curcuma longa* (Turmeric) rhizome were collected from the local Super market. Dry spices (100 gm) were crushed and sieved through mesh cloth to get the fine powder. Powdered spices were soaked in 200ml of distilled water and were kept at room temperature for 24 hours, then were filtered using Whatman no. 1 filter paper. The filtrate was heated at 40-50°C using water bath, until thick paste is formed. The thick paste was considered as 100% concentration of extract. These extracts were stored at 4°C in refrigerator.\[16\]

Animals

Eight to ten weeks old male mice (*Mus musculus*) weighing about 25gm, procured from National Institute of Nutrition, Hyderabad, were used in this study. The mice were housed in polypropylene cages in a well ventilated room and were provided with standard pellet diet (M/S Lipton India limited) and water ad libitum. They were maintained under controlled conditions of temperature and light.

For Sperm Morphology Assay, in the first test three doses of *Curcuma longa* extract (CRE) i.e. 150, 250 and 350 mg/kg body weight were administered for seven days and control group of animals were fed only with physiological saline. In the second test animals were divided into groups and group I ,group II, group III, group IV and were given 16 mg/kg body wt Adriamycin (ADR), 150(CRE) +16(ADR) mg/kg body wt, 250(CRE)+16(ADR) mg/kg body wt,350 (CRE) +16g (ADR) mg/kg body wt respectively. *Curcuma longa* extract was dissolved in double distilled water and administered as a single dose in 2 ml per mouse for 7days prior to adriamycin administration. The drug was supplied by Apollo Pharmacy, Hyderabad. For each dose group three animals were used and for each animal 500 sperms were screened. The animals were given adriamycin intravenously in a single dose within 24 h interval. The control group of mice received physiological saline simultaneously.

Sperm Morphology Assay

All the exposed and control animals were sacrificed on last day of fifth week by cervical dislocation after exposure to adriamycin. Spermatogonial stage which is exposed to drug would reach to cauda epididymis after undergoing a series of changes during the process of maturation to give rise to sperm. Animals were sacrificed and dissected out for both testis and cauda epididymis, which were removed and placed in petridish containing 0.9% NaCl (hypotonic physiological saline) solution. The cauda epididymis were teased thoroughly to release the sperm and stained with 1% aqueos eosin for about 20-30 minutes. A drop of
sperm suspension was smeared on a clean slide. One thousand sperms per animal were scored for each group for the presence of sperm shape abnormalities.\textsuperscript{[17]}

**Statistical analysis**

For statistical evaluation of the experimental data Chi-Square test was performed. To determine the frequency of various sperm head abnormalities about 500 sperms were scored for each animal for the presence of amorphous, banana shaped, hammer headed, pin headed sperms etc. All the data was analysed for the significance of experimental versus control data using the Chi-square test.

**RESULTS AND DISCUSSION**

Cytogenetic procedures are very helpful to evaluate the clastogenic activity of chemicals which are chief components of chemotherapy drugs that are responsible for genotoxicity. The Sperm Morphology Assay functions as an important and sensitive indicator in assuming reproductive genotoxicity. They can be used to estimate the spermatogenic impairment, fertility and heritable genetic alterations. In the present investigation Sperm Morphology Assay was conducted following the criteria of Wyrobek and Bruce (1975). As numerous types of mutations can lead to abnormal sperm morphology, this test is treated as more sensitive test in detecting germ cell mutagens than other germinal mutagenicity assays.\textsuperscript{[18]} Sperm morphology assay is also said to provide a quantitative technique for locating genetic impairment in male germ line cells. In our laboratory numerous drugs have been tested for the induction of sperm head abnormalities and published elsewhere.\textsuperscript{[19]}

Several \textit{in vitro} and \textit{in vivo} experimentations revealed the therapeutic potential of curcumin an active ingredient of \textit{Curcuma longa} and defensive effects of curcumin. However in our investigation we intended to evaluate the modulating effects of crude extract of \textit{Curcuma longa} against adriamycin induced genotoxic impairment. An effort has been made in the present experimentation to evaluate whether such toxic effects induced by adriamycin are counteracted or counter balanced by administration of \textit{Curcuma longa} rhizome extract.

In the current examination the higher occurrence of sperm abnormality induced by adriamycin is a measure of genetic impairment produced at the spermatogonial stage of the mouse germ cells. In the current examination the results on the incidence of sperm head abnormalities in \textit{Curcuma longa} rhizome extract treated animals are represented in Table 1. There is a slight rise in the percentage of abnormal sperms that is 2.00\% in controls to 2.60\%,
2.93%, 3.13% in 150, 250 and 350 mg/kg body weight *Curcuma longa* rhizome extract treated animals respectively. The variances in the incidence of sperm abnormalities were found to be statistically insignificant when compared between control and *Curcuma longa* rhizome extract treated groups. Hence, the results clearly indicate the antimutagenic nature of *Curcuma longa* rhizome extract. The results on the percentage of sperm abnormalities in *Curcuma longa* rhizome extract +Adriamycin treated mice are presented in Table 2. There is a rise in the percentage of abnormal sperms that is 13.00% when adriamycin alone is administered in a group compared to control group that is 2.00%. There is a gradual reduction in the percentage of abnormal sperms that is 11.66%, 10.13%, 8.46% in 16 mg/kg B. wt ADR, 150 +16 mg/kg B. wt, 250 +16 mg/kg B. wt and 350 +16 mg/kg B wt. *Curcuma longa* rhizome extract +Adriamycin treated animals respectively when compared to adriamycin alone treated group that is 13.00%. There is a significant reduction in the occurrence of sperm head abnormalities in groups primed with *Curcuma longa* rhizome extract (**P<0.01, *P<0.05)**. There is an increase in the percentage of inhibition in the percentage of abnormal sperms with increase in the dose of *Curcuma longa* rhizome extract in *Curcuma longa* extract +Adriamycin treated animals. The percentage of inhibition by *Curcuma longa* rhizome extract in 150 +16 mg/kg B.wt, 250 +16 mg/kg B.wt and 350 +16 mg/kg B wt. doses of *Curcuma longa* rhizome extract +Adriamycin treated animals is 12.12 %, 26.06 % and 41.21% respectively (Table.2; Graph.1). Various types of Sperm Abnormalities were shown in Fig(1).

The main genetic effect of adriamycin and related compound is binding to DNA. It is known that adriamycin and other anthracyclines induce peroxide production in various tissues. Cellular enzymes are capable of converting adriamycin into free radical metabolites, adriamycin cytotoxicity may be mediated by free radicals derived from this drug.\[20\] The present results are comparable with that of Meistrich et al\[21\] who has studied sperm production and fertility in male mice treated with adriamycin (ADR) at 6 or 8 mg/kg body weight. Testicular sperm production and epididymal sperm counts were markedly decreased after adriamycin treatment. Gradual recovery of counts occurred but sperm counts have not reached control levels even more than 1 year after treatment. Epididymal sperm morphology indicated that adriamycin treatment induced morphological abnormalities throughout the test; the frequencies of sperm with detached tails and the frequencies of sperm with morphologically abnormal heads continued to raise for about 2-3 fold above control.
Similarly Mestrich et al.,\textsuperscript{22} stated that the mutagenic effect of Adriamycin on mouse spermatogonial stem cells is tested by examination of spermatocyte chromosome and of dominant lethality transmitted through the spermatozoa. The effect of adriamycin on mutation, cytotoxicity and sperm head abnormalities were compared with that of radiation. The cytotoxic effect of 6GY of gamma-radiation on stem spermatogonia was equivalent to about 4-5 mg/kg b.wt. adriamycin. Chromosomal translocations were observed in 6% of spermatocyte of mice treated with adriamycin.

*Curcuma longa* is an obligatory food additive and an individual in his diet can consume 1-5g/day of powdered form of *Curcuma longa* (Turmeric) which acts as a cleaning agent renders to protect against many diseases.\textsuperscript{23}

Several medical properties have been attributed to *Curcuma longa* Linn. Rhizome of Haridra is known to possess therapeutic activities and has been used by medical practitioners as an anti-diabetic,\textsuperscript{24} hypolipidemic,\textsuperscript{25} anti-inflammatory, anti-diarrhoal,\textsuperscript{23} hepatoprotective,\textsuperscript{24} anti-asthmatic,\textsuperscript{25} and anti-cancerous drug. Haridra is widely used in cosmetology.\textsuperscript{26} The fresh juice of Haridra is considered to be anthelmintic.\textsuperscript{27} Rhizome is used to treat cough and cold. Hepatoprotective,\textsuperscript{28} Neuroprotective activity,\textsuperscript{29} Alzheimer’s disease symptoms characterized by inflammation and oxidation were also eased by curcumin’s powerful antioxidant and anti-inflammatory properties.\textsuperscript{30} Chemoprotective activity and anti-cancer activity,\textsuperscript{31} anti allergic activity,\textsuperscript{32} antidermatophytic activity.\textsuperscript{26} Curcumin has also been shown as an immunostimulant and immunorestor in *in vivo* this mechanism may also participate in cancer preventive activity.\textsuperscript{33} Curcumin has revealed strong antioxidant activity and studies have shown curcumin to reduce oxidative stress. Hence it can conclude that antioxidant such as curcumin protects the body from damage to free radicals.

### Table: 1: Frequency of sperm head abnormalities in mice administered with various doses of *Curcuma longa* rhizome extract (CRE).

<table>
<thead>
<tr>
<th>Treatment dosage in mg/kg/bw</th>
<th>Normal sperms</th>
<th>Abnormal sperms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Control</td>
<td>1470</td>
<td>98.00</td>
</tr>
<tr>
<td>150 CRE</td>
<td>1461</td>
<td>97.40</td>
</tr>
<tr>
<td>250 CRE</td>
<td>1456</td>
<td>97.06</td>
</tr>
<tr>
<td>350 CRE</td>
<td>1453</td>
<td>96.86</td>
</tr>
</tbody>
</table>

The p>0.05 level, hence the difference is considered to be statistically insignificant.
Table 2: Frequency of sperm head abnormalities in Adriamycin (ADR) treated mice primed with *Curcuma longa* rhizome extract (CRE).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment &amp; Dose (mg/kg/bw)</th>
<th>Normal sperms</th>
<th>Abnormal sperms</th>
<th>% of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Control</td>
<td>1470</td>
<td>98.00</td>
<td>30</td>
</tr>
<tr>
<td>Group II</td>
<td>16 mg/kg ADR</td>
<td>1305</td>
<td>87.00</td>
<td>195</td>
</tr>
<tr>
<td>Group III</td>
<td>150 CRE + 16 ADR</td>
<td>1325</td>
<td>88.33</td>
<td>175</td>
</tr>
<tr>
<td>Group IV</td>
<td>250 CRE + 16 ADR</td>
<td>1348</td>
<td>89.86</td>
<td>152</td>
</tr>
<tr>
<td>Group V</td>
<td>350 CRE + 16 ADR</td>
<td>1373</td>
<td>91.53</td>
<td>127</td>
</tr>
</tbody>
</table>

Table 3: $\chi^2$ values for the differences in the frequency of abnormal sperms in adriamycin treated animals primed with *Curcuma longa* rhizome extract (CRE).

<table>
<thead>
<tr>
<th>Treatment: Dose (mg/kg bw)</th>
<th>$\chi^2$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vs 16 ADR</td>
<td>129.230**</td>
</tr>
<tr>
<td>16 ADR vs 150 CRE + 16 ADR</td>
<td>1.113</td>
</tr>
<tr>
<td>16 ADR vs 250 CRE + 16 ADR</td>
<td>5.748*</td>
</tr>
<tr>
<td>16 ADR vs 350 CRE + 16 ADR</td>
<td>15.617**</td>
</tr>
</tbody>
</table>

The ** $p<0.01$ level, hence the difference is considered to be statistically very significant. 
* $p<0.05$ level, hence the difference is considered to be statistically significant.
Various types of Sperm Abnormalities (Fig: 1)

Normal Sperm Banana shaped Sickle shaped Hammer headed.

Tail bent Neck bent Head detached Pin headed

CONCLUSION
Animals when treated with various doses of *Curcuma longa* rhizome extract revealed antimutagenic effect and the percentage of sperm head abnormalities were nearly equal with that of control values. In the present investigation Adriamycin, a chemotherapy drug presented significant rise in the frequency of abnormal sperm morphology, but when animals primed with *Curcuma longa* rhizome extract, a significant inhibition of genotoxicity was observed in adriamycin treated animals. Thus the overall results indicate the modulating
nature of *Curcuma longa* rhizome extract against drug induced damage in swiss albino male mice.

**ACKNOWLEDGEMENTS**

One of the authors J. Karuna Kumari is thankful for Award of fellowship under the scheme of Maulana Azad National Fellowship for Minority Students to University Grants Commission, New Delhi and Prof. K. Pratap Reddy, Head, Department of Zoology, Osmania University, Hyderabad for providing Laboratory facilities.

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