

# A comparative study of radioprotection by four Indian medicinal herbs against genotoxicity induced by sub-lethal gamma irradiation in Swiss albino mice

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**Background:** Synthetic radio-protective agents like aminothiols are toxic and FDA approved agent amifostine is no exception. Some Ayurvedic herbs have shown radioprotective potential. This study was carried out to test and compare the radioprotective potential of *Curcuma longa* (CL) *Tinospora cordifolia* (TC), *Zizyphus mauritiana* (ZM) and *Ocimum sanctum* (OS) against 2Gy gamma irradiation in Swiss albino mice.

**Materials and Methods:** Adult Swiss albino mice from random breed colony were divided in to 6 groups (n=9), sham-control (SCT), radiation control (RACT), and four herb + radiation groups respectively. All except SCT were exposed to whole body 2 Gy of gamma radiation in a teletherapy unit and SCT was sham exposed on day 7 of herb pretreatment (200mg/kg-bw orally by gavage). Chromosomal studies from the bone marrow of femur by routine metaphase preparation after colchicine treatment were done in 3 animals from each group at 24, 72 and 168 hours after exposure. **Results:** All four herbs showed significant radio-protective effects at 24 hrs. OS, TC and ZM showed nearly similar activity while CL showing the lowest activity. However the effects at 72 and 168 hrs showed highest protection by CL followed by ZM ≥ TC > OS respectively suggesting that the well studied OS was less effective at 72 and 168 hrs. **Conclusion:** All the four herbs showed radio-protective potential with different efficacies at different time interval. Iran. J. Radiat. Res., 2008; 6 (1): 19-30

**Keywords:** Radioprotection, chromosomal-damage, *curcuma longa*, *ocimum sanctum*, *tinospora cordifolia*, *zizyphus mauritiana*.

## INTRODUCTION

The development of radio protective agents has been the subject of intense research in

view of their potential utility in a radiation environment like space exploration, radiotherapy units, nuclear power plants reactor accidents like Chernobyl in 1986 and even nuclear warfare <sup>(1)</sup>. Up to eighty percent of cancer patients need radiotherapy either for curative or palliative purpose. In order to obtain better tumor control with a higher dose, the normal tissues should be protected and this could be achieved by various techniques as well as radioprotective agents. Thus the role of radioprotective compounds is very important in clinical radiotherapy. Aqueous free radicals are generated by radiation energy in the cells and their reactions with DNA, RNA, and organelle cause cell dysfunction, mortality, mutagenesis or carcinogenesis <sup>(2)</sup>. This process of cell damage is modulated by various factors like presence of oxygen, sulfhydryl compounds and level of cellular protective enzymes like superoxide dismutase (SOD), glutathione peroxidase (Gpx) and catalase <sup>(3, 4)</sup>. Evidently, rapidly dividing cells like epithelial and hemopoietic system are prone to early and marked damage to chromosomes as well as other organelle due to higher content of oxygen and water with a higher level of free radical

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generation on impact of radiation energy.

Numerous compounds of synthetic and natural origin like- antioxidants, sulfhydryl compounds, ACE inhibitors, cytoprotective agents, metalloelements, immunomodulators, lipopolysaccharides and prostaglandins, vitamin A, C, E, and DNA binding ligands have been tested by *in vivo* and *in vitro* models with little success <sup>(5)</sup>. The established therapy after radiation injury is limited to prevention of infection and general supportive care. Synthetic radio protectors like amifostine, ethiophos and gammaphos are highly toxic at radioprotective dose <sup>(6)</sup>. Many Ayurvedic, Chinese and other herbs as well have been used to treat free radical mediated ailments so it is logical to expect radioprotective potential in them <sup>(7)</sup>. Orientin, Vicenin and Ursolic acid in *Ocimum sanctum* (OS) have shown radio protective activity comparable to amifostine without toxic side effects. Herbs like *Centella asiatica* Linn, *Ginkgo biloba* Linn *Hippophae shamnoides*, *Mentha piperita* Linn. *Panax ginseng* and *Curcuma longa* and some poly herbal formulas with Rasayana herbs have been studied for their radioprotective action in different models <sup>(7, 8)</sup>. However most of the studies have been carried out for different concentrations of different types of extracts varying between as low as 5mg/kg body weight (bw) in case of TC to 1% fortification of diet by curcumin coming to 2.5g/kg-bw in different setups with different parameters <sup>(9, 10)</sup>. In the present experiment we have taken *Curcuma longa* (CL), *Tinospora cordifolia* (TC), *Ocimum sanctum* (OS) and *Zizyphus mauritiana* (ZM) for studying their comparative radioprotective efficacy. These are four most common, cheap and easily available, identifiable and standardizable herbs from Ayurvedic arena. Extracts can not represent the whole herb most of the time and some active fraction may be lost, so high quality crude was selected for present experiment. Dosage of herbs was extrapolated from human dose and radiation dose i.e. 2Gy so that the results of the study could be used for a prospective clinical trial.

To best of our knowledge nobody has done a comparative study of four herbs for radioprotective potential in similar setup and the indigenous variety of herb *Zizyphus mauritiana* (Indian Ber) has been tested for the first time.

*Curcuma longa* (CL) belonging to family Zingiberaceae, a plant that is known as Haldi in India and Turmeric in English, has demonstrated a wide spectrum of therapeutic effects such as anti inflammatory, antioxidant, anti mutagenic, antitumor, antifungal, antiviral, antibacterial, antispasmodic and hepatoprotective. Recently its potential utility in acquired immune deficiency syndrome (AIDS) was demonstrated <sup>(11)</sup>. No acute toxicity in mice was observed on administration of turmeric powder with dose as high as 10g/kg-bw <sup>(12)</sup>. The lowest published toxic oral dose for mouse is 13650 mg/kg for 13-weeks the toxic effect being changes in liver weight <sup>(13)</sup>.

*Ocimum sanctum* (OS) belongs to family Labiatae, a plant that is known as Tulsi in India and Holy Basil in English is known to have adaptogenic activity <sup>(14)</sup>. OS contains a volatile oil consisting of about 70% eugenol as well as methyl eugenol and caryophyllene. Other constituents with likely pharmacological activity include the triterpenoids ursolic acid, rosmarinic acid, oleanic acid; flavonoids apigenin and luteolin; alkaloids; saponins; phenylpropane glucosides and tannins. The seeds contain a fixed oil containing five fatty acids, including about 17% linolenic acid and just over 50% linoleic acid <sup>(15)</sup>. It has numerous pharmacological activities like hypoglycemic, antistress, immunomodulatory, analgesic, antipyretic, anti-inflammatory, antiulcerogenic, antihypertensive, CNS depressant, hepatoprotective, chemopreventive, radioprotective, antitumor and antibacterial <sup>(16, 17)</sup>. OS ethanolic extract 200 mg/kg-bw for 30-days in rats and 500 mg/kg-bw for 15-days in mice did not produce any toxic side effects <sup>(18, 19)</sup>. Doses up to 4g/kg-bw for 14-days did not produce any toxicity or mortality in rats <sup>(20)</sup>. The LD<sub>50</sub> of aqueous extract of OS in mice was found to be ≥5g/kg-bw <sup>(21)</sup>.

*Tinospora cordifolia* (TC) belongs to family Menispermaceae; a large climbing shrub,

growing throughout tropical India; and popularly known as Giloya in Hindi and *Tinospora* in English. It contains tinosporine, tinosporide, tinosporaside, cordifolide, cordifol, heptacosanol, clerodane furano diterpene, diterpenoid furanolactone tinosporidine, columbin and  $\beta$ -sitosterol. The aqueous extract of guduchi stem has shown the presence of arabinogalactan that showed immunological activity. The bitter principle present shows adaptogenic antispasmodic, anti-inflammatory, antipyretic, anti-neoplastic, hypolipidemic, hypoglycemic, antioxidant, immunopotentiating and hepatoprotective properties<sup>(17, 22)</sup>. It is used in general debility, digestive disturbances, loss of appetite and fever in children. It is also an effective immunostimulant<sup>(23)</sup>. 400 mg/kg-bw of aqueous extract of TC given per oral (PO) to Swiss albino mice for 60 days did not produced any significant toxic effect<sup>(24)</sup>. LD<sub>50</sub> for TC PO in Swiss albino mice was found to be 2650 (2209-3901) mg/kg<sup>(25)</sup>.

*Zizyphus mauritiana* (syn. *Zizyphus jujuba* L.) (ZM), belonging to family Rhamnaceae is a plant of very common occurrence. It grows wild in forests and also on wastelands throughout India. In India it is commonly known as 'Ber' and in English it is known as Indian berry. Pharmacologically active compounds from seeds include jujubojenin, jujubosides-A1 B and C, acetyljujuboside B1, protojujubosides A, B, & B1<sup>(26, 27)</sup>. The seeds are traditionally used for insomnia and anxiety. The active compounds are reported to have inhibitory effects on hippocampal formation *in vivo* and *in vitro* probably through its anti-calmodulin action<sup>(28)</sup> and a potent immunological adjuvant activity<sup>(27)</sup>. ZM is reported to have very low toxicity when taken orally, in mice and rats; a huge single dose of 50 g/kg-bw produced no toxic symptoms and a daily dose of 20 g/kg-bw for 30 days did not produced toxic reactions. No side effects were reported<sup>(29)</sup>.

## MATERIALS AND METHODS

### Plant material

Different varieties of CL have been widely

cultivated in different parts of India and they contain 1.5-4% of active principle curcumin. Variety *Selum* found to have ~4% of curcumin content was selected for present experiment. The dried rhizome of *Selum* was purchased from local market and ground to make fine powder. The leaves and inflorescence of dark variety of OS known as *Krishna Tulsi* cultivated in the Botanical garden of botany department of Veer Narmad South Gujarat University was collected and shade dried to make fine powder. The crude powder of TC known as *Giloya* or *Amrita* was purchased from local pharmacy (ASFA- GMP-ISO 9001-2000 certified). Fruits of *Randeri* variety of ZM cultivated in Dist. Surat were collected and seeds were ground to make a fine powder. CL, OS and ZM were identified and certified by Prof. Dr. M.H.Parabha (HOD) a taxonomist at Dept. of Biosciences, Veer Narmad South Gujarat University, Surat. The voucher specimen for CL, OS and ZM - No.s MRA/0501, MRA/0502 and MRA/0503 respectively were deposited in the herbarium of Bapalal Vaidya Botanical Research Centre of the same institute.

Standardization was done by HPTLC fingerprinting using curcumin (Konark, herbals Pvt. Ltd.), ursolic acid (Anchrom, India), tinocordin (Wockhardt Pharmacy) and saponin (Anchrom, India) as standard for CL, OS, TC and ZM respectively for confirming the quality of crude powders.

### Animals

Adult Swiss female albino mice (35-45g) were provided by SPAN Diagnostic Ltd, and the experiment was carried out in animal house of SPAN research centre. The permission was obtained from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and its guidelines were followed throughout the experimental procedure. Mice were housed in polypropylene boxes, in a controlled environment (temperature 23 $\pm$  2°C and 12hr dark and light cycle) with standard laboratory diet and water *ad libitum* in the animal house of the same institution.

### **The study groups**

Swiss albino mice were divided into six groups (n=9). One group sham control (SCT); one group radiation control (RACT); four herb + radiation groups viz. [RA +CL, RA + OS, RA +TC, RA +ZM], all the herb + RA groups were pre-treated with their respective herbs for seven days before exposure to the radiation.

### **Irradiation**

Unanesthetized mice were restrained in a ventilated Perspex box and except SCT group all other groups were given 2 Gy  $\gamma$ -radiation at mid point by AP/PA whole body dose at the rate of 150 cGy /min from Co<sup>60</sup> tele-therapy unit at a distance of 80 cm from the source at Lion's Cancer Research Center, Surat. SCT group was sham exposed to mimic the possible effect of stress of the procedure.

### **Dosage**

The dose of herbal treatment, extrapolated from the human dose was 200 mg/kg body weight by using Rubner's hypothesis and Meeh's formula  $S=kW^{0.667}$ , where S is surface area in square centimeters, k is a species specific constant (10.5 for mice) and W is mass expressed as body weight in grams (30-32). All the herbal treatment groups were given the herb once daily orally and SCT and RACT group Bengal gram powder by gavage for seven days and then till sacrifice.

### **Hematological study**

Hematological indices were studied at Doctor's Clinical Lab. on Sysmax K-1000 Cell Counter (Japan) on day 0, day 7 i.e. before and after administration of herbs for seven days and 24, 72 and 168 hrs after whole body irradiation at the time of sacrifice.

### **Chromosomal study**

After the exposure to  $\gamma$ -radiation, three animals from each group at 24, 72 and 168 hours were selected randomly for chromosomal study. Animals were given 0.2 ml/100 g-bw colchicines (50mg% w/v) i.p. three hours before they were sacrificed by cervical dislocation. Bone marrow from the

femur bone was aspirated and the cells were centrifuged, treated with hypotonic salt solution (0.56% KCl), fixed in methanol-acetic acid (3:1) and metaphase plates were prepared by the air drying method (modified G-banding technique) (33). Slides were stained with 3% Giemsa (Sigma) and aberrations of 100 metaphases were scored from each mouse on Axioskop-2 FS plus Carl-Zeiss fluorescent microscope equipped with Metasystem Group Inc. software: Isis (version 5.0) and Ikaros (version 5.0) at Gene Lab with the help of a qualified geneticist.

### **Statistical analysis**

All the values were expressed as mean  $\pm$  SEM. The data were statistically analyzed by One Way ANOVA followed by post hoc Tukey's test for HSD. P values  $\leq 0.05$  were considered significant.

## **RESULTS**

After seven days of herb treatment, no toxic effects were observed in terms of alteration in behavior, activity, diet intake, micturition-defecation pattern and body weight except a nonsignificant reduction of 2.5 to 3.5 g body weight in RA+CL group.

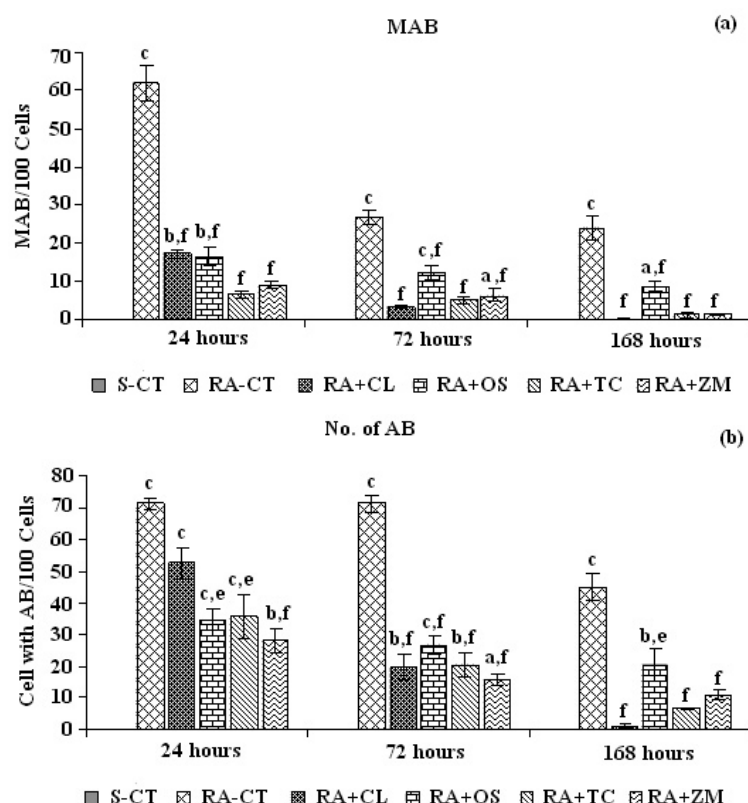
After whole body 2 Gy  $\gamma$ -irradiation, the animals in RACT group showed some effects like hair loss, weight loss, diarrhea and mild irritability in 5-7 days. None of these effects were observed in any of the herb treated group except a mild degree of hair loss in RA+OS group.

As seen in table 1 the SCT group had 0.66% of aberrant cells which consisted of chromatid gap and break. No chromosome type gap (chr-m-gap) or break (chr-m-brk) or complex aberrations like dicentric rings etc. were noted. Irradiation significantly increased the percentage of cells with any aberration (AB) and cells with more than six aberrations (MAB) at all three time intervals as shown in figure 1 with a decreasing trend in incidence along with time. Figure 2 a and b depicts the examples of various types of abnormalities seen in a multiple abnormal

**Table 1.** Shows different types of chromosomal aberrations (Mean  $\pm$  SEM) in mouse bone marrow after 24 (1A), 72 (1B) and 168 (1C) hours of exposure to 2Gy whole body gamma radiation.<sup>a</sup>p  $\leq$  0.05; <sup>b</sup>p  $\leq$  0.01; <sup>c</sup>p  $\leq$  0.001- compared with SA-CT;<sup>d</sup>p  $\leq$  0.05; <sup>e</sup>p  $\leq$  0.01; <sup>f</sup>p  $\leq$  0.001- compared with RA-CT.

chr= chromosome; cht= chromatid; brk= break

| (1A)         | ABERRATIONS / 100-CELLS    |                       |                |                   |                           |                    |                       |               |
|--------------|----------------------------|-----------------------|----------------|-------------------|---------------------------|--------------------|-----------------------|---------------|
| 24-hrs       | CHROMOSOME TYPE ABERRATION |                       |                |                   | CHROMATID TYPE ABERRATION |                    |                       |               |
|              |                            |                       | Ring           |                   |                           |                    | Chromatid-interchange |               |
| Groups       | chr <sup>m</sup> -gap      | chr <sup>m</sup> -brk | acentric       | dicentric         | cht-gap                   | cht-brk            | tri-radiate           | qua-radiate   |
| S-CT         | 0                          | 0                     | 0              | 0                 | 0.07 $\pm$ 0.3            | 0.07 $\pm$ 0.3     | 0                     | 0             |
| RA-CT        | 119 $\pm$ 6.8c             | 74.33 $\pm$ 6.5c      | 4.7 $\pm$ 0.7b | 7.7 $\pm$ 0.9c    | 162 $\pm$ 15.2c           | 171.3 $\pm$ 21.2c  | 4.3 $\pm$ 0.9c        | 1.3 $\pm$ 0.3 |
| RA+CL        | 45.3 $\pm$ 3.5c f          | 40.3 $\pm$ 9.4b e     | 3.3 $\pm$ 0.9a | 4.7 $\pm$ 0.3 c e | 118.7 $\pm$ 17c           | 128 $\pm$ 9.2c     | 1.7 $\pm$ 0.7d        | 0.3 $\pm$ 0.3 |
| RA+OS        | 29 $\pm$ 2.3b f            | 22.7 $\pm$ 1.9f       | 3.3 $\pm$ 0.7a | 0 f               | 38.7 $\pm$ 2.2 f          | 113 $\pm$ 8.4 c d  | 0 f                   | 0             |
| RA+TC        | 19.7 $\pm$ 1.8f            | 20 $\pm$ 3.1f         | 2.7 $\pm$ 0.9  | 2.7 $\pm$ 0.3 b f | 42.3 $\pm$ 7.8 f          | 88.3 $\pm$ 6.5 b e | 1 $\pm$ 0 e           | 0.3 $\pm$ 0.3 |
| RA+ZM        | 22.7 $\pm$ 7.2a f          | 23.3 $\pm$ 2.6c       | 0e             | 0 f               | 50 $\pm$ 3.8 a f          | 45.3 $\pm$ 10 f    | 0 f                   | 0.3 $\pm$ 0.3 |
| ANOVA-F(cal) | 71.95                      | 17.38                 | 6.09           | 53.3              | 25.43                     | 14.66              | 13.05                 | 2.9           |
|              |                            |                       |                |                   |                           |                    |                       |               |
| (1B)         | ABERRATIONS / 100-CELLS    |                       |                |                   |                           |                    |                       |               |
| 72-hrs       | CHROMOSOME TYPE ABERRATION |                       |                |                   | CHROMATID TYPE ABERRATION |                    |                       |               |
|              |                            |                       | Ring           |                   |                           |                    | Chromatid-interchange |               |
| Groups       | chr <sup>m</sup> -gap      | chr <sup>m</sup> -brk | acentric       | dicentric         | cht-gap                   | cht-brk            | tri-radiate           | qua-radiate   |
| S-CT         | 0                          | 0                     | 0              | 0                 | 1 $\pm$ 0.6               | 0.3 $\pm$ 0.3      | 0                     | 0             |
| RA-CT        | 90.7 $\pm$ 8.5c            | 30.3 $\pm$ 4.2c       | 3 $\pm$ 1a     | 4.3 $\pm$ 0.9c    | 114.3 $\pm$ 7.7c          | 100 $\pm$ 9.1c     | 0                     | 0             |
| RA+CL        | 10 $\pm$ 1.5f              | 2.3 $\pm$ 0.3f        | 0 d            | 0 f               | 50 $\pm$ 6.7 c f          | 10 $\pm$ 1.5 f     | 0                     | 0             |
| RA+OS        | 17 $\pm$ 2f                | 20.3 $\pm$ 4.9b       | 2.3 $\pm$ 0.9  | 1.7 $\pm$ 0.7 d   | 38 $\pm$ 4.7 b f          | 45.3 $\pm$ 1.9 c f | 1 $\pm$ 0.6d          | 0             |
| RA+TC        | 0f                         | 12.7 $\pm$ 1.8e       | 0 d            | 0 f               | 41.7 $\pm$ 6.7 b f        | 27.7 $\pm$ 4.4 a f | 0                     | 0             |
| RA+ZM        | 11.3 $\pm$ 0.9f            | 10.7 $\pm$ 1.9e       | 0.7 $\pm$ 0.3e | 0 f               | 25.7 $\pm$ 3.5 f          | 42 $\pm$ 7.5 b f   | 0                     | 0             |
| ANOVA-F(cal) | 86.08                      | 11.61                 | 5.09           | 14.68             | 33.43                     | 34.85              | 3                     | -             |
|              |                            |                       |                |                   |                           |                    |                       |               |
| (1C)         | ABERRATIONS / 100-CELLS    |                       |                |                   |                           |                    |                       |               |
| 168-hrs      | CHROMOSOME TYPE ABERRATION |                       |                |                   | CHROMATID TYPE ABERRATION |                    |                       |               |
|              |                            |                       | Ring           |                   |                           |                    | Chromatid-interchange |               |
| Groups       | chr <sup>m</sup> -gap      | chr <sup>m</sup> -brk | acentric       | dicentric         | cht-gap                   | cht-brk            | tri-radiate           | qua-radiate   |
| S-CT         | 0                          | 0                     | 0              | 0                 | 0                         | 0                  | 0                     | 0             |
| RA-CT        | 48 $\pm$ 5.7c              | 26.3 $\pm$ 2.4c       | 2.3 $\pm$ 1.5  | 0.7 $\pm$ 0.3a    | 80 $\pm$ 16c              | 76.7 $\pm$ 7.3c    | 0                     | 0             |
| RA+CL        | 0f                         | 0.7 $\pm$ 0.3f        | 1 $\pm$ 0      | 0d                | 0 f                       | 0 f                | 0                     | 0             |
| RA+OS        | 10.7 $\pm$ 1.7f            | 11.7 $\pm$ 1.5c f     | 1.7 $\pm$ 0.9  | 0d                | 25 $\pm$ 6.4 e            | 45 $\pm$ 8.7 c e   | 0                     | 0             |
| RA+TC        | 0f                         | 3.7 $\pm$ 0.3f        | 0              | 0d                | 23.3 $\pm$ 6.9 e          | 11.7 $\pm$ 1.7 f   | 0                     | 0             |
| RA+ZM        | 10 $\pm$ 2.1f              | 4.3 $\pm$ 1.2f        | 0              | 0d                | 21.7 $\pm$ 2 e            | 19.7 $\pm$ 1.8f    | 0                     | 0             |
| ANOVA-F(cal) | 49.9                       | 55.81                 | 1.83           | 4                 | 12.74                     | 34.58              | -                     | -             |



**Figure 1.** a) Multiple Aberrations (MAB) i.e.  $\leq 6$  aberrations / metaphase. MAB / 100 cells (Mean  $\pm$  SEM) in mouse bone marrow after 24, 72 and 168 hours of exposure to 2Gy whole body gamma radiation. b) Total no. of metaphase having any aberration per 100 metaphase scored (Mean  $\pm$  SEM) in mouse bone marrow after 24, 72 and 168 hours of exposure to 2Gy whole body gamma radiation.

<sup>a</sup>p  $\leq 0.05$ ; <sup>b</sup>p  $\leq 0.01$ ; <sup>c</sup>p  $\leq 0.001$ - compared with SA-CT;

<sup>d</sup>p  $\leq 0.05$ ; <sup>e</sup>p  $\leq 0.01$ ; <sup>f</sup>p  $\leq 0.001$ - compared with RA-CT.

No. of AB = number of cells with any aberrations (Mean  $\pm$  SEM) per 100 cells scored.

metaphase in RACT group and a karyotype with deletion of sex chromosome respectively. The same findings were reflected in analysis of different types of aberrations like chrm-gap, chrm-brk and acentric- dicentric rings; and chromatid type of aberrations like gap (cht-gap), break (cht-brk), tri-radiate and quadra-radiate type of exchange as depicted in table 1 at 24 hours scoring of metaphases. Though chromatid exchange and rings were minimal at 72 and 168 hours study of cells; chromatid and chromosome type gaps and breaks remained significantly higher in RACT group.

In all the herb- pretreated groups, the incidence of AB and MAB were significantly lower at all the three post irradiation time

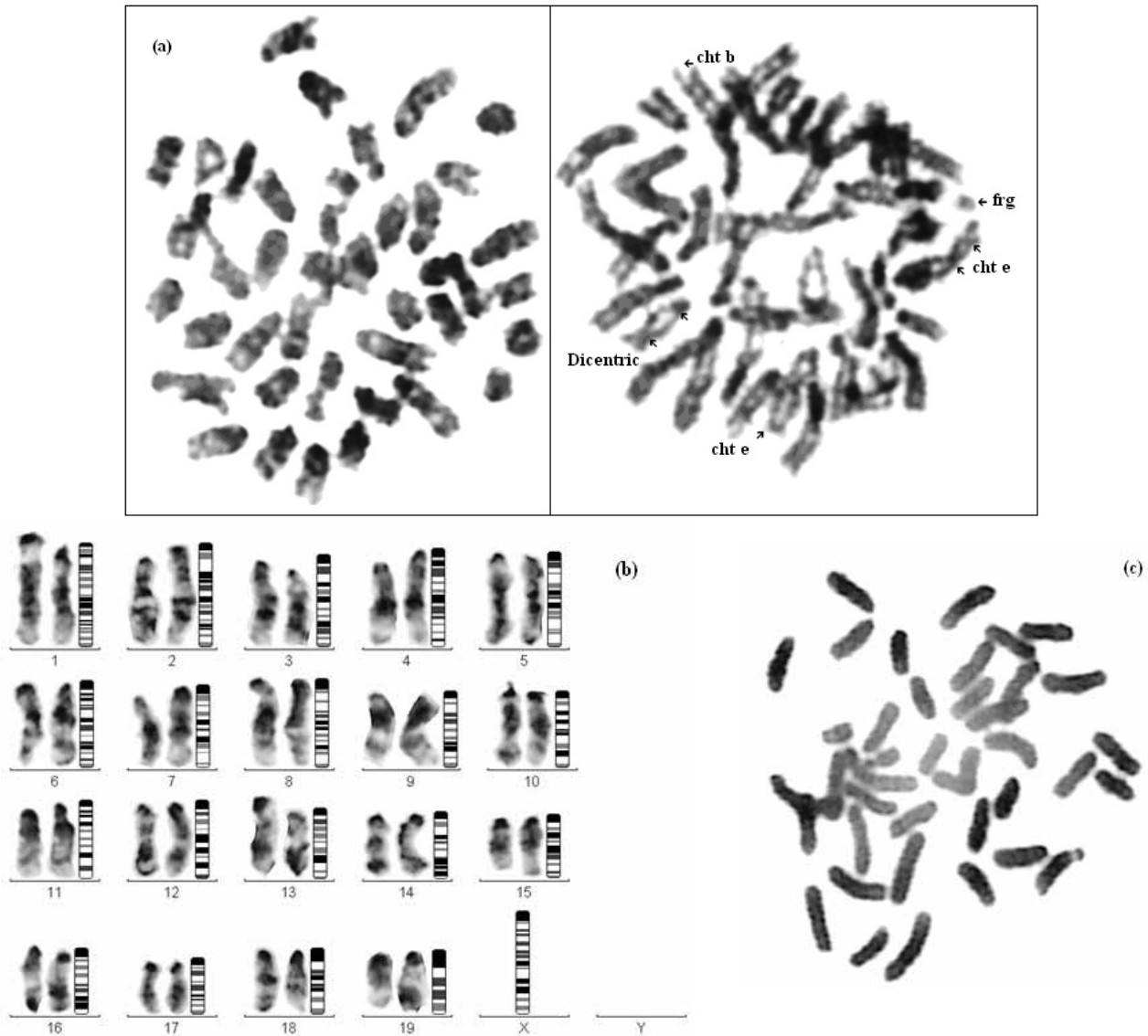
intervals except in RA+CL at 24 hrs with insignificant decrease in AB (figure 1). Similarly as seen in table 1, cht-gap, cht-brk and acentric ring were not significantly lower in RA+CL as compared to RACT at 24 hrs. RA+OS, RA+TC and RA+ZM showed markedly reduced incidence of AB and MAB (figure 1) along with increase in normal metaphase (figure 2c) at 24 hrs. At 72 and 168 hrs the decrease in incidence of aberrations in RA+OS though significant as compared to RACT; it was not as spectacular as in case of RA+CL, RA+TC and RA+ZM. Significant persistence of cht-brk, cht-gap and chrm-brk at 72 hrs and cht-brk and chrm-brk at 168 hrs were observed in RA+OS. In RA+ZM cht-brk and in RA+TC cht-gap and cht-brk both remained significantly persisted at 72 hrs. None of the aberration persisted significantly at 168 hrs in all except RA+OS.

Hematological data at day 0 and day 7 after herb treatment did not reveal any significant change. Post-irradiation hematological data at 24, 72 and 168 hrs failed to show any consistent pattern or a significant ANOVA. The cause may be base line variability with a wide margin of

normality resulting in increased variance and repeated measure method could not be adopted as the animals were to be sacrificed at three time intervals for chromosomal preparation (data not shown).

## DISCUSSION

The herbs tested in present experiment have excellent toxicological profile as described earlier. No toxicity was observed at the end of 7 days of pre-treatment in terms of food intake, bowel and micturition pattern, agility and normal activity. Genotoxicity would be expected with ionizing radiation or chemical mutagens only and it is not common



**Figure 2.** a) Metaphases with multiple chromosomal aberrations in RA-CT group. b) Metaphase with deletion of Sex chromosome. c) Normal Metaphase.

Cht b = chromatid break; cht e = chromatid exchange; frg = fragment

to find it by normal food item or simple medicinal Ayurvedic herbs so no chromosomal study was done before irradiation of the animals in herb treated groups. Hematological data at the end of 7 days of herb treatment did not cause any significant changes in hemoglobin, RBC, total WBC and platelet counts.

Most of the herbal and synthetic radioprotective agents render their maximal effect at dose reaching near its maximum tolerated dose (MTD) <sup>(34)</sup>. However the herbs in present experiment have very high MTD

and the idea behind the experiment was to test a dose that would be parallel to known human dose as these herbs have been in vogue for different purposes since centuries in India. So Rubner's hypothesis <sup>(30)</sup> (metabolic rate being proportional to body area rather than body weight) was adopted and Meeh's formula <sup>(31)</sup> was used to calculate body area of mice and accordingly the dose per bw decided. However Rodents are fast metabolizer of drugs, and metabolic activities like  $\beta$ -glucuronidation, acetylation, deacetylation etc. are several times higher than that of

humans in many tissues <sup>(35)</sup> and that was also taken in to account.

When an organism is exposed to ionizing radiation, many systems are affected with maximum effects over rapidly dividing cells like hematopoietic stem cells and mucosal epithelium and morbidity or mortality depends upon initial damage at cellular level that depends upon presence of oxygen, sulfhydryl group and cellular protective enzymes like SOD, catalase etc <sup>(3,4)</sup>. The survival of animal would depend on multisystem efforts of the organism to combat infection and maintain vital functions and integrity of the body during the period of repair at cellular level. Antiemetic, anti-inflammatory, antimicrobial, anti-oxidant, haemopoietic stimulant, immunostimulant, metal chelation and wound healing activities would contribute to radioprotective potential of an agent <sup>(7)</sup>. However preservation at cellular level would depend primarily on prevention of genotoxicity at cellular level and this would imply that presence of cellular protective agents or a change in cellular milieu interior in favor of cell against radiation assault must be achieved before the exposure; so pretreatment design was preferred. Then continuation of the treatment was done until sacrifice for helping the reparative efforts by the experimental animal. The results of such an experiment could logically be used for future human trials if planned exposure like therapeutic radiation or known exposures to radiation in other situations are to be tested. There are controversial views regarding the effects of low dose exposure to radiation which may help to improve health and survival as well as cause a decrease in incidence of infections, sterility, cancer deaths and premature deaths according to "The Radiation Hormesis Thesis" that recognizes large dose of ionizing radiation as inhibitory and harmful while small dose elicit opposite results by stimulatory effect and may be beneficial <sup>(36)</sup>. However 2Gy dose for whole body irradiation was selected because it is sub-lethal and preferred dose for a fraction of conventional fractionation radiotherapy for treating

cancers in humans <sup>(37)</sup>.

In RACT group AB remained nearly similar at 24 and 72 hours and reduced at 168 hours while there was a marked reduction in MAB between 24 and 72 hours. This would be explained by either a natural repair process or elimination of MAB with proliferation of AB with generation of new AB. It is a known fact that chromosomal abnormality is expressed in later generations of AB cells and that is the basis of lymphocyte culture technique adopted for chromosomal abnormality studies in human <sup>(38)</sup>. At 168 hours the MAB reduced a little while AB reduced further and this may represent the natural repair process.

Pre-treatment with CL caused no significant reduction in AB at 24 hours though it did prevent the MAB to a significantly lower level giving an evidence of protective activity. At 72 and 168 hours the MAB are virtually eliminated either due to elimination or repair or both while AB falls gradually to nil. Though curcumin is considered to be the active principle of *Curcuma longa*, it contains many other biologically active molecules with anti oxidant, anti-inflammatory activities <sup>(39, 40)</sup>. With oral route the bioavailability of pure curcumin is very limited <sup>(41, 42)</sup> so administration of high quality crude herb by oral route was preferred. Also there is no approved parenteral preparation of curcumin is available for humans. Curcumin can prevent damage and help the repair by anti oxidative, free radical scavenging and anti-LPO activity while increasing the level of catalase, SOD glutathione peroxidase significantly along with GSH level <sup>(38, 43)</sup>. On the other hand it can induce apoptosis in critically damaged cell by activation of caspase 3 and 8 <sup>(44)</sup>. In radiation exposed cells, p53 plays an important role in repair process by arresting the cell cycle in G1 <sup>(45)</sup>. Curcumin up regulates the p53 expression and induces G2/M cell cycle arrest to enhance the repair and regulates apoptosis thereby preventing mutagenesis. <sup>(46)</sup> Also curcumin has been shown to sensitize squamous cell carcinoma cell lines against radiation <sup>(47)</sup>.

Protective action for normal cells and sensitizing effect on malignant cells would make it an ideal adjuvant agent for patients undergoing radiotherapy for cancer treatment.

In RA+OS both AB and MAB were controlled significantly at 24 hours but further decrease at 72 and 168 hours was not as spectacular. Complex aberration and both chromatid and chromosome types of aberration persisted at 168 hours suggesting a weaker action of either repair or elimination after 24 hours. OS contains flavonoids orientin and vicenin having radioprotective activity but other compounds like ursolic acid, methyl eugenol and rosmarinic acid may be contributory to radioprotective effect <sup>(7, 48)</sup>. Aqueous extract of leaves of OS had been shown to protect against radiation at 50 mg/kg-bw i.p. without any toxicity and with comparable efficacy to 300 mg/kg-bw of amifostine- an FDA approved synthetic thiol compound radioprotector with marked systemic toxicity <sup>(49, 50)</sup>. The probable mechanism of action was shown to be inhibition of OH radical induced deoxyribose degeneration <sup>(51)</sup>.

In RA+TC, AB and MAB both were significantly lower at 24 hrs and reduced further at 72 hours. MAB were virtually eliminated while AB remained at significantly lower level at 168 hours. TC was distinctly superior to CL at 24 hours; to OS at all time intervals and comparable to CL and ZM at 72 and 168 hours. TC has been shown to have anti-oxidant properties by preventing reactive oxygen species (ROS), reactive nitrogen species (RNS), lipid-peroxidation; and by enhancing the activity of SOD and catalase <sup>(52)</sup>. It increased survival in Swiss albino mice against sublethal dose of radiation <sup>(9)</sup>. TC has also been shown to counter the gamma irradiation induced deleterious effects on immune system cells and their activities like adherence, spreading and phagocytosis; cytokines level in serum and antioxidant potential of plasma <sup>(53)</sup>. Like CL it has also a radiosensitizing action on malignant cells in addition to protective effect on normal cells hence it is a promising

agent for a prospective human trial for patients under going radiotherapy for treatment of cancer <sup>(54)</sup>.

RA+ZM group showed a significant reduction in AB at 24, 72 and 168 hours with a gradual decline - being best at 24 and 72 hours and comparable with TC at 168 hours. MAB remained comparable to TC at all three time intervals. Complex aberrations were totally eliminated by 168 hours while few chromosome and chromatid type of gaps and breaks remained but they were insignificant compared to SCT. Overall it could be inferred that ZM showed consistent efficacy at all the time intervals studied.

ZM, the Indian variety of it, has never been tested as a single herb for radioprotective potential. In Dukes Handbook of Medicinal Herbs the Chinese jujube has been shown to have radioprotective activity along with multitudes of other activities <sup>(55)</sup>. *Z. jujuba* has been tested as a part of poly herbal formula of traditional Chinese medicine (TCM) Kuei-Pi-Tang for various activities including radioprotective potential <sup>(56)</sup>. Kim *et al.* (1999) showed the *Jujuba* seed extract to protect the spleen cells against radiation in *in vitro* experiment <sup>(57)</sup>. A natural product named CKBM containing water extract of jujube seeds along with other four herbs and Baker's yeast has been shown to arrest the cell cycle at G2/M checkpoint suggesting its potential to stop proliferation helping the repair process and induce apoptosis helping eliminate the damaged cells with mutated genetic material. This activity would suggest anti-tumor as well as chemopreventive potential to be there in ZM <sup>(58)</sup>. ZM has been shown to have immunostimulant, hepatoprotective and anti-inflammatory activity as evaluated by its capacity to inhibit COX-2 <sup>(17, 59)</sup>.

It has triterpinoides having anti-complementary activity <sup>(60)</sup> and various cyclopeptides and peptide alkaloids having inhibitory effect on calmodulin-dependant calcium-ATPase and phosphodiesterase <sup>(61)</sup>. These activities may be contributory to its immunomodulatory, sleep inducing and relaxation enhancing effects and that might indirectly help the repair process at cellular

level as well as organism level.

## CONCLUSION

All the 4 herbs have radioprotective potential with different efficacies and pattern at 3 different time periods, probably because different molecules might work by several different mechanisms and metabolize by different pathways at their own speed in different organs or tissues. A prospective study of the effects of four herbs on human cancer cell lines would further clear their significance and help design a human trial for patients undergoing radiotherapy for different kind of malignant tumors so that the side effects of radiation is kept to a minimum with maintained or enhanced effects on the tumor. As there is no single ideal radioprotective agent available, combination of multiple molecules having different mechanisms of action might prove superior.

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