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Protective Effects of *Solanum Lycopercicum* Fruit Extract In cyclophasphamide Induced Genotoxicity in Germ Cells of Mice

KEYWORDS: *Solanum Lycopercicum* fruit extract (SLFE), cyclophosphamide, Chromosomal aberrations.

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ABSTRACT *In the present investigation the antimutagenic effects of solanum lucopercicum fruit extract has been evaluated against lead nitrate induced genotoxicity. Single IP administration of solanum lycopercicum fruit extract at various doses i.e. 250, 500 and 1000 mg /kg . When treated individually did not induce chromosomal aberration in germ cells of mice. A single intraperitoneal injection of 50mg/kg of cyclophosphamide induced significant increase in the percentage of chromosomal aberrations in germ cells of mice. However after co administration of three doses of Solanum lycopercicum fruit extract there was a dose dependent decrease in the % of chromosomal aberrations was observed. Thus the results clearly indicate the preventive effects of solanum lycopercicum fruit extract against cyclophosphamide induced genotoxicity in germ cells of mice. Therefore the data indicate that SL fruit extract is a safer dietary component in cancer chemo preventive strategy.*

INTRODUCTION

Cyclophosphamide (CPM) is a well-known bifunctional alkylating agent widely used in cancer chemotherapy and expresses its genotoxicity when metabolically activated (Fleming, 1997). It is extensively used for the treatment of various cancers as well as an immunosuppressant in organ transplantation, rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, and other benign diseases (Perini et al, 2007**;** Uber et al, 2007**)**. According to the International Agency for Research on Cancer (IARC), CPM is widely used as reference mutagen and has been classified as carcinogenic for animals and humans (IARC, 1987). Reactive metabolites of CPM chemically alkylate DNA as well as protein, producing cross-links which are responsible for its cytotoxic effect. Normal tissues injury or damage is the major limitation of using CPM, which gives rise to numerous side effects, CPM treatment also results in the production of reactive oxygen species (ROS), which cause peroxidative damage to kidney and other vital organs. Antineoplastic and toxic effects like necrosis, apoptosis, and oncosis of CPM are linked with two active metabolites, i.e., phosphoramide and acrolein. Further Tripathi and Jena (2008) reported CPM to be toxic in germ cells of mice, it has also been reported that CPM treatment for non-Hodgkin's lymphoma leads to the induction of secondary cancers in bladder and kidney. The important factor for therapeutic and toxic effects of CPM is the requirement of metabolic activation by hepatic microsomal cytochrome P mixed functional oxidase system (Eckhardt1 et al, 2008**).** On activation, CPM generates active alkylating metabolites such as 4 hydroxycyclophosphamide, acrolein, and aldophosphamide mustard, which hamper with cellular DNA synthesis in fast dividing cells and ultimately lead to cell death (Bagley & Bostick, 2001; Muneeb et al, 2012). Further cyclophosphamide induced chromosomal aberrations in somatic and germ cells of mice (Rudrama devi. & Keshava Rao, 2007).

Solanum lycopersicum (tomato) is an important vegetable in India, Several epidemiological and experimental studies suggested the preventive role of lycopene, a active constituents of Solanum lycopersicum reduction in the risk of several different types of cancer. Such as cancers of the lung, stomach, prostate gland, cervix, breast, oral cavity, pancreas, colorectum, and esophagus (Giovannucci & Clinton, 1998; Michaud et al, 2000; Norrish et al, 2000; Rao & Agarwal, 1998; Freudenheim et al, 1996**)** Dietary lycopene comes primarily from tomatoes, although apricots, guava, watermelon, papaya, and pink grapefruit are also significant sources. Tomatoes are the best source of lycopene. A population-based case control study found that lycopene from *Solanum lycopersicum* (tomato) based foods was associated with a small reduction in risk for prostate cancer. High concentration of lycopene in prostate tissues resulted in a nearly three-fold increase in programmed cell damage among cancer cells. It has been suggested that lycopene supplements may benefit those with prostate cancer (Giovannucci & Clinton, 1998). In animal studies the antitumour effect of Lycopene was reported in S180 tumor which inhibited the growth of S180 tumor (Pan et al, 2004). Lycopene did not caused direct maternal or developmental toxicity in rats or rabbits at dosages as high as 2000 or 3000 mg/kg/day (Christian et al, 2003). Therefore, we have made to study the antimutagenic effect of Solanum lycopersicum fruit extract using the chromosomal in germ cells of mice.

Methodology:

Chemical

Cyclophosphamide was purchased from Merck Chemical Ltd. Other Reagent grades chemical were procured locally.

Extract Preparation

The identification of the plant *Solanum lycopersicum* (family: *Solanacae*) was done by botanist Prof. Prathiba devi, Department of Botany, Osmania University, Hyderabad, Andhra Pradesh, India. The *S. lycopersicum* fruit were collected. The

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pieces of fruits were taken and cut in to small pieces. After that paste was taken in a separating funnel and added double distilled water and extracted with double distilled water by refluxing for 36 hrs. at 60°C. On the day of experimentation, the desired amount of powder was dissolved in double distilled water for the final administration.

Animal and Treatment

The study was conducted on random 6-7 weeks old and 24- 28 gm body weight male *Swiss albino* mice. They were maintained under controlled conditions of temperature and light (light: dark, 12 hrs: 12 hrs.). They were provided standard mice feed and water *ad libitum*. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC, Ref. No. 2157/225/2006). For micronucleus test, three dose of *S. lycopersicum* i.e. 250, 500 and 1000 mg/kg body weight were administered. *S. lycopersicum* extract were dissolved in double distilled water and administered to mouse 24 hours prior to cyclophosphamide administration.

Slightly modified procedure of Evans et al., (1964) was used to prepare the slides to evaluate the action of Cyclophasphamide, *tomato fruit extract* on different stages of spermatogenesis. Both control and treated groups of animals were sacrificed after 28 days of exposure to test compounds with a view to cover spermatogenetic cycle of mouse (Hannan-alva, 1965; Adler, 1984). These sampling time yields valuable data on which stages of spermatogenesis are more sensitive to the test agent and on the persistence and reversibility of the induced damages (Wyrobek & Bruce, 1978). In the present study the air drying technique of Evans et al., (1964)[21] was employed with slight modifications to study the effect of test compounds on meiotic cells of mice.

In the present study on dose effect relationship the animals were injected intraperitonially with various doses of Cyclophosphamide 50 mg/kg body wt. and various doses of *tomato fruit extract* 250, 500 & 1000mg/kg body wt. administered orally for 7 consecutive days. Control group of animals were also maintained simultaneously. According to the timing of spermatogenesis (Oakberg, 1956) after Cyclophasphamide, and *tomato fruit extract* treatment i.e. or 28th day the animals were sacrificed by cervical dislocation.

All the animals were sacrificed by cervical dislocation on 28th day. Animals were dissected out for testis and kept in 0.9% physiological saline. Tunica albugenia, the membrane covering the testes was removed carefully and the tubules were transferred to another Petri dish containing 5ml of 1.2% tri-sodium citrate. The tubules of the testes were teased in hypotonic solution. The cell suspension was collected in clean centrifuge tubes and incubated at 37°C for 45minutes. After incubation the tubes were centrifuged for 10minutes at 1000 rpm. The supernatant was discarded and to the pellet 5ml of freshly prepared pre-chilled fixative (3:1 methanol and acetic acid) was added drop wise from the sides of the centrifuge tubes and immediately dispersed the cell suspension by aspirating several times with a Pasteur pipette. The tubes were left undisturbed for 10 minutes at room temperature. This step was repeated 4 to 5 times. In the final change the cells were suspended in 0.5ml of fresh fixative 3 to 4 drops of cell suspension were dropped from a height of 30-35cms on clean grease free, pre-chilled slides with a pipette. The slides were stained with 2% giemsa for 8 -10min Gin inures and later they were rinsed in double distilled water and allowed to dry.

The slides were screened and a total of 100 well spread spermatocytes at the diakinesis of metaphase-1 of meioses were observed per animal for the presence of various types of chromosomal aberrations like structural and numerical aberrations in control and treated groups and micro photographed.

RESULTS:

The data on the incidence of chromosomal aberrations in germ cells of mice are depicted in table 1-4 and graphically illustrated in graphs 1-3.

The frequencies (%) in the controls recorded were 3.00% of abnormalities and the percentages of chromosomal aberrations were 3.20, 3.40 & 3.60 after administration of 250, 500 & 1000 mg/kg tomato fruit extract respectively (Table- 1 & 2). Change in the chromosomal number were recorded as autosomal univalent in controls was 1.20 when compared to treated mice were 1.20, 1.40 & 1.20 in after administration of 250, 500 & 1000 mg/kg tomato fruit extract treated groups. Sex chromosomal univalents in controls were 1.40 with that of tomato fruit extract treated mice was 1.40, 1.40 & 1.60 with 125, 500 & 1000 mg/kg body wt. TFE respectively. Among polyploids, aneuploidy results in controls were 0.40 when compared to that of ginger extract mice was 0.40, 0.40 & 0.40 respectively. Polyploids were 0.20, 0.20 & 0.40 in all the treated groups. No translocations were observed in any of the groups. The differences in the frequencies in the chromosomal aberrations between controls and treated mice were analyzed using X^2 test and the results were found to be insignificant (P>0.05, Table-1 and Graph 1).

Table 1: Frequency of Chromosomal Aberrations recorded in germ cells of mice with various doses of tomato fruit extract.

Treatment	Normal metaphases scores %	Abnormal metaphases scores
Control	485(97.00)	15(3.00)
250mg/kgTFE	484(96.80)	16(3.20)*
500 mg/kgTFE	483(96.60)	17(3.40)*
1000 mg/kgTFE	482(96.40)	18(3.60)*

The values in the parentheses are percentages.
*p>0.01

Table 2: Classification of CA's in germ cells of mice treated with tomato fruit extract.

Treatment dose (mg/kg)	Changes in chromosomal number (%)				Structural changes (%)
	Autosomal univalents	Sex chromosomal univalents	Aneuploids	Polyploids	Translocations
Control	6(1.20)	7(1.40)	2(0.40)	0(0.00)	0(0.00)
250 mg/kg TFE	6(1.20)	7(1.40)	2(0.40)	1(0.20)	0(0.00)
500 mg/kg TFE	7(1.40)	7(1.40)	2(0.40)	1(0.20)	0(0.00)
1000mg/kg TFE	6(1.20)	8(1.60)	2(0.40)	2(0.40)	0(0.00)

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The values in the parentheses are percentages.

Among the non-primed groups the controls have shown only 3.00% of abnormal metaphases when compared to 20.80% in CP alone administered mice. There was a significant decrease in the percentage of abnormal metaphases in mice primed with tomato fruit extract (250, 500 & 1000 mg/kg body wt.) as 16.20, 14.20 & 12.00 respectively for various concentrations (Table- 3 & 4). The frequency of autosomal univalents were 1.00 in control increased to 7.20 in Cyclophosphamide alone treated mice, however the frequency of CA's decreased to 5.60, 5.20 and 4. 200 in 250 + 50, 500 + 50, 1000 +50 mg/kg Tomato extract + cyclophosphamide treated group of animals. The percentage of sex chromosomal univalents were 5.20 in cyclophosphamide treated animal and decreased to 4.20, 4.00, 3.60 after the administration of 250 + 50, 500 + 50, 1000 +50 mg/kg tomato extract + cyclophosphamide treated group of animals. The frequency of polyploidys were 4.00 in cyclophosphamide treated group of animals and decreased to 3.20, 2.60, 2.20 in 250 + 50, 500 + 50, 1000 +50 mg/kg tomato extract + cyclophosphamide treated group of animals. The percentage of aneuploids were 2.80 in cyclophosphamide treated animals and the frequencies were 2.00, 1.60 & 1.20 after the administration of 250 + 50, 500 + 50, 1000 +50 mg/kg Tomato fruit extract + cyclophosphamide treated group of animals. No translocations were observed in control animals were as 1.60 % was seen in cyclophosphamide treated groups and decreased to 1.20, 0.80 & 0.80 in 250 + 50, 500 + 50, 1000 +50 mg/kg tomato fruit extract + cyclophosphamide treated group of animals. The differences in the frequencies of the chromosomal abnormalities between the controls and treated mice were analyzed by X^2 test and the results were found to be significant (P<0.01, Table- 3 & 4 and represented in graphs 2 &3).

Table 3: Frequency of CA's recorded in CP induced genotoxicity primed with tomato fruit extract.

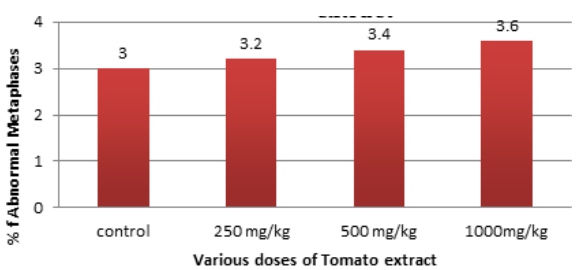
Groups	Dose	Normal metaphases	Abnormal metaphases	% of Inhibition
Group- I	Control	485(85.00)	15(3.00)	
Group -II	50mg/kgCP	396(79.20)	104(20.80)	
Group-III	250 +50mg/kgGE+ CP	419(83.80)	81(16.20)	22.11
Group-IV	500+50mg/kg GE +CP	429(85.80)	71(14.20)	31.73
Group- V	1000+50mg/kg CP	440(88.00)	6012.00)	43.30

The values in the parentheses are percentages. P<0.05.

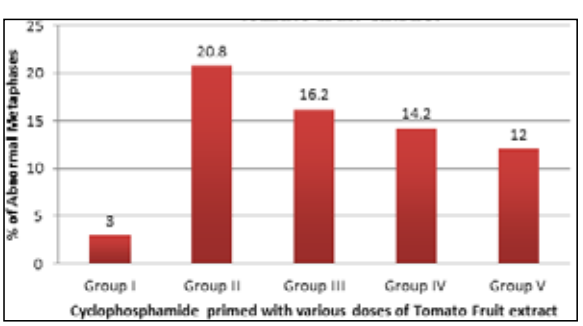
Table 4: Classification of chromosomal aberrations in germ cells of mice treated with cyclophosphamide & primed with tomato fruit extract(TFE).

Treatment dose in (mg/kg)	Changes in chromosomal number (%)				Structural changes(%)
	Autosomal univalents	Sex chromo-somal univalents	Polyp-loids	Aneu-ploids	Translocations
Control	5(1.00)	7(1.40)	2(1.40)	1(0.20)	0(0.00)
CP50mg/kg	36(7.20)	26(5.20)	20(4.00)	14(2.80)	8(1.60)
TFE 250+50mg/kgCP	28(5.60)	21(4.20)	16(3.20)	10(2.00)	6(1.20)
TFE 500+50mg/kgCP	26(5.20)	20(4.00)	13(2.60)	8(1.60)	4(0.80)
TFE 1000+50mg/kgCP	21(4.20)	18(3.60)	11(2.20)	6(1.20)	4(0.80)

The values in the parentheses are percentages.



Graph 1: Frequency of chromosomal Aberrations in germ cells of mice with various doses of Tomato extract



Graph 2: Frequency of CA's recorded in cyclophosphamide induced genotoxicity primed with tomato fruit extract

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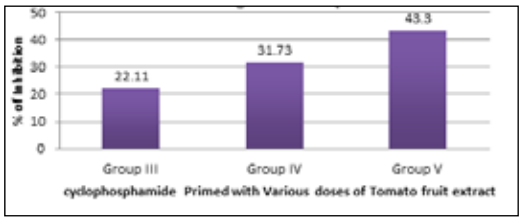

Graph 3: Percentage of Inhibtion by Tomato fruit extract Against Cyclophosphamide induced genotoxicity

Discussion:

Cytogenetic evaluation of chromosomal damage in germ cells induced by a toxicant or mutagen is of specific significance. Since the gametes transmit the effects from one generation to another generation. The studies on behavior of sex-chromosomes and autosomes are of cardinal importance since the inherited anomalies like congenital malformations, still births, neonatal deaths, repeated abortions and other genetic disorders may arise due to the mutations which are induced in germ cell line.

The present results are in accordance with the Hu et al., (2005); induction of chromosomal aberrations in germ cells of mice and micro nucleus in bone marrow cells were observed in dose dependent manner in CP treated mice . Choudhury et al., (2002) worked for the cytogenetic toxicity after a single intraperitoneal exposure of three different doses (5, 10 and 15mg/kg body wt.) of 5-FU and its transmission in the male germ line cells of Swiss mice was assessed. At 24hrs post-treatment each of the doses of 5-FU induced statistically highly significant number of chromosomal aberrations, mostly random chromatid breaks, in the spermatogonial cells with maximum aberrations in the lowest dose

A study revealed marked protective role of both vitamin E and ginger oil on cyclophosphamide induced male gonadal dysfunction. The later represented by altered male gonadal weight, disturbed sperm quality parameters, decreased testosterone level, disturbed oxidative stress and lipid peroxidation markers in addition to altered spermatogenesis, testicular histology and increased incidence of apoptosis among germ cells,. The findings of the present study pass in accordance with similar studies which previously reported that cyclophosphamide induced testicular androgenic and gametogenic dysfunction (Fukushima et al, 2005; Selvakumar et al, 2004).

Naturally occurring antioxidants have been extensively studied for their capacity to protect organisms and cells from oxidative damage. Many plant constituents including *S. lycopersicum* and Lycopene appear to be potent antimutagens and antioxidants. Lycopene did not cause direct maternal or developmental toxicity in rats or rabbits at dosages as high as 2000 or 3000 mg/kg/day (Freudenheim et al, 1996) and Synthetic crystalline lycopene provides an alternative to extracts of naturally occurring lycopene for use in dietary supplements and functional foods. BASF Lycopene 10 CWD and Lyco Vit 10% formulated products each contain approximately 10% synthetic lycopene. These products were evaluated for toxicological and behavioural effects during a 13-week oral dosing study with male and female Wistar rats. The no-observed-adverse-effect level (NOAEL) for this study was concluded to be 3000 mg/kg body weight per day for both Lycopene CWD and Lyco Vit (Mellert et al, 2006). The present data demonstrate that In *S.lycopersicum* fruit extract was dose dependent inhibition of micronuclei induced by CP in mouse bone marrow cells. *S. lycopersicum*, when tested for mutagenic effect at various test dose levels, failed to induce micronuclei. Pre-treatment with lycopene had significantly reduced the frequency of CP-induced bone marrow micronuclei . The similar kinds of earliar studies have also been reported that several naturally occurring compounds exhibited antimutagenic activity. These include Indole-3-carbinol (I3C) (Agrawal & Kumar, 1999).The non mutagenic effect of Lycopene active constituent of *S. lycopersicum* extract has been also observed also in MNNG-induced micronuclei formation and chromosomal aberration test system (Velmurugan et al, 2004). Further the protective role of lycopene on bisphenol a induced changes in sperm characteristics, testicular damage and stress in rats was reported (Agrawal et al, 2009). Lycopene prevented chemically-induced DNA and chromosome damage and tumor-promoting activity in liver cells through antioxidant activity and inhibition of growth factors and signaling pathways (Tamilselvan et al, 2013). In a clinical trial, lycopene supplementation (30 mg/day for 2 months) had beneficial effects in healthy women with a high risk of breast cancer but not in breast cancer survivors (Scolastici et al, 2008).

Carotenoids, as potential antioxidants, are well known as highly efficient scavengers of singlet oxygen ($1O_2$) and other excited species. During $1O_2$ quenching, energy is transferred from $1O_2$ to the lycopene molecule, converting it to the energy-rich triplet state. Trapping of other ROS, such as OH, NO_2 or peroxynitrite, in contrast, leads to oxidative breakdown of the lycopene molecule. Thus, lycopene may protect in vivo against oxidation of lipids, proteins, and DNA (Voskuil et al. 2008). Lycopene has been shown to have the highest antioxidant activity among the carotenoids in cell protection against hydrogen peroxide and nitrogen dioxide radical components. In addition, lycopene has been reported to attenuate oxidative stress and exert anticancer effects both in vitro and in vivo (Stahl & Sies 2003). Previous studies reported that oral lycopene therapy in men with idiopathic infertility provided an improvement in male infertility, especially in sperm characteristics (Jonker et al, 2003). A rational mechanism for potential anticarcinogenic and antimutagenic effects of β-carotene and other carotenoids is their ability to scavenge free radicals that cause oxidative DNA damage (Gupta & Kumar 2002). These findings are in agreement with the data of the present study. The protective effects of lycopene against CP induced abnormal sperm rates may be attributed to the antioxidant properties of lycopene. These observations might also indicate that lycopene has protective role on cyclophosphamide induced genetic damage in germ cells of mice. The protective effect of lycopene is because of pigment principally responsible for the characteristic deep red colour of ripe tomato fruits and tomato products. Lycopene has been shown to have the highest antioxidant activity among the carotenoids in cell protection against free radicals (Cohen, 2002). It may also contribute to the prevention or amelioration of oxidative damage to cells and tissues both in vivo and in vitro (Yonar & Sakin, 2011). Moreover, studies have suggested that the anticancer effects of lycopene are related to their effectiveness as antioxidants (Velmurugan et al, 2002; Dias et al, 2010; Waliszewski & Blasco, 2010). Antioxidant activity of tomato and tomato products was related to lipophilic constituents such as b-carotene, lycopene, and organic phenolic compounds (Karakaya &Yılmaz 2007). Tomatoes and related tomato products are the major sources of lycopene, and are also considered an important source of carotenoids in the human diet (Wu et al, 2004). Our laboratory has been published on the protective effects of ascorbic acid and

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garlic extract against lead induced genotoxicity in *invivo* and *invitro* test system (Rudrama Devi et al, 2003; Lakshmi Soujanya et al, 2008).

CONCLUSION

Animals when treated with various doses of *Solanum lycopercicum* fruit extract showed non mutagenic nature and the percentage of chromosomal aberration in germ cells of mice were equivalent with that of control values. There was an increase in the incidence of chromosomal aberrations in cyclophosphamide treated group when compared to the control Group. There was a significant decrease in the percentage of chromosomal aberration in germ cells of mice when cyclophosphamidewas primed with various doses of *Solanum lycopercicum* fruit extract. Thus Tomato fruit extract showed protective effects against the cyclophosphamide induced genotoxicity in germ cells of mice. Hence *Solanum lycopercicum* fruit extract supplementation is a safer dietary component in chemotherapeutic strategy.

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REFERENCE Adler ID (1984). Oxford: IRI. pp. 275-306 Agrawal, R.C., Jain, R., Wasim Raja, Ovais, M., (2009). Asian Pacific Journal of Cancer Prevention.10: 379-381. Agrawal, R.C., Kumar, S. (1999): Toxicology Letters,106:137-141. Bagley C, Bostick F. 2001. Cancer Res 33:226–235. Choudhry, Tandra Das and Archana Sharma (2007). CancerLettersVolume 121, Issue 1, Pages 45-52. Christian MS, Schulte S, Hellwig J. (2003) Food Chem Toxicol. Vol- 41(6) pp- 773-833. Cohen LA. (2002). Exp. Biol Med, 227:864–8. Dias, M.C., Vieiralves, N.F., Gomes, M.I., Salvadori, D.M., Rodrigues, M.A., Barbisan, L.F., 2010. Food Chem. Toxicol. 48, 772–780. Eckhardt1 S., A. J. Prata1 , P. Seibert2 , K. Stebel1 , and A. Stohl1 Atmos. Chem. Phys., 8, 3881–3897, 2008 Evans Ep, Breckon G, Ford Ce. (1964). Cytogenetics. 3:289-94. Fleming RE., 1997. Pharmacotherapy17:1465–1545. Freudenheim, J.L,Marshall, J.R., Vena,J.E.,Laughlin,R., Brasure, J.R., Swanson, M.K.,Nemoto,T., Graham, S., (1996).J.Natl. cancer inst. 88,340- 348. Fukushima T, Yamamoto T, Kikkawa R, Hamada Y, Komiyama M, Mori C, Horii I. (2005). JToxicol Sci. Aug; 30(3):195-206. Giovannucci E, Clinton SK. (1998) Proc.Soc.Exp. Biol.Med. Vol. - 218: pp-129-139. Gupta NP, Kumar R. (2002).Lycopene Int Urol Nephrol, 34:369 –72. Hannan-alva A (1965). Adv. Genet. 13:157-226. Hu Q, Xu J, et al. (2005).. CancerLett. 220 (1): 29-35. IARC monographs on the evaluation of carcinogenic risks to humans. Supplement 7, 1987. Jonker D, Kuper CF, Frail N, Estrella A, Rodrigues Otero C. (2003). Regul Toxicol Phamacol, 37:396– 406. Karakaya S., N. Yılmaz J. Sci. Food Agric., 87 (2007), pp. 2342–2347. Lakshmi Soujanya B., D Madhavi and K Rudrama Devi; Bull. Env. Sci. Vol.XXVII (2nd Issue), pp.141-146, 2008. Mellert, W., Deckardt, K., Gembardt, C., Schulte, S.,Ravenzwaay, B. van, Slesinski, R. S., (2006) Food and Chemical Toxicology, CABI Abstract, April. Michaud DS, Feskanich D, Rimm EB, Graham AC.,Walter CW. And Edward Giovannucci (2000) American of clinical Nutrition;Vol - 72: pp - 990-997. Muneeb U. Rehman, Mir Tahir, Farrah Ali, Wajhul Qamar, Abdul Lateef, Rehan Khan,Abdul Quaiyoom, Oday-OHamiza and Sarwat Sultana . 2012 . Mol Cell Biochem. Jun; 365(1-2):119-27 Norrish AE, Jackson RT, Sharpe SJ, Skeaff CM., (2000) American Journal of Epidemiol. Vol–151, pp-119- 123. Oakberg EF. (1956). Am JAnat. 99(3):391-413. Pan H, Jiang X, Wan L, Na L, Wang J. (2004). Vol - 33: pp – 456-457. Perini P, Calabrese M, Rinaldi L, Gallo P. 2007 . Expert Opin Drug Saf 6:183–190. Rao AV, Agarwal S. (1998). Nutrient Cancer. Vol – 31, pp - 199-203. Rudrama devi. K and K. Keshava Rao. 2007. Indian journal of multidisciplinary research 3(1):169-172. Rudrama Devi. K, D. Madhavi and P.P reddy; Indian J. Environ. Toxicol. 13(1):1-4, 2003. Scolastici C, Alves de Lima RO, Barbisan LF, Ferreira AL, Ribeiro DA, Salvadori DM. (2008) Toxicol In Vitro. 22(2):510-514. Selvakumar E, Mythili Y, Sudharsan PT, Varalakshmi P. (2004). Chem Biol Interact. 151(1):13–19. Stahl W, Sies H. (2003).Antioxidant activity of carotenoids, Mol Aspects Med, 24:345–51. Tamilselvan. P, Bharathiraja.K, Vijayaprakash.S and Balasubramanian .M.P. (2013) Int J Parma and bio sci oct ; 4(4): (p)131-143. Tripathi DN, Jena GB. (2008). Toxicology; 248:96–103. Uber WE, Self SE, Van Bakel AB, Pereira NL. 2007. Am J Transplant 7:2064–2074 Velmurugan, B., Bhuvaneswari, V., Nagini, S., 2002. Fitoterapia 73, 604–611. Velmurugan, B., Santhiya, S.T., Nagini, S., (2004). Pol J Pharmacol, 56: 241-5. Voskuil DW, Vrieling A, Korse CM, et al. Nutr Cancer . 2008;60(3):342-353. Waliszewski, K.N., Blasco, G., 2010. Salud Publica Mex. 52, 254–265. Wu, K., Erdman Jr., J.W., Schwartz, S.J., Platz, E.A., Leitzmann, M., Clinton, S.K., DeGroff, V., Willett, W.C., Giovannucci, E., 2004. Cancer Epidemiol. Biomar. Prev. 13, 260–269. Wyrobek AJ, Bruce WR. (1978). Exp. Morphol. 1971; 25:223-236 Yonar, M.E., Sakin, F., 2011. Pest. Biochem. Physiol. 99, 226–231.