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# Investigation on antimutagenic activity of *Carthamus tinctorius* oil in mice

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## ABSTRACT

The present study was designed to investigate the anti-mutagenic effect of Carthamus tinctorius oil (CTO) on cyclophosphamide-induced mutagenicity and its impact on oxidative stress. Cyclophosphamide and its metabolites can bind DNA, causing damage that may result in chromosome breaks and icronucleus formation. Incorporation of antimutagens in our diet is the best way to minimize the effect of mutagens. Micronuclei assessment from bone marrow and peripheral blood was used in the present study to assess the damage to DNA. Cyclophosphamide in a single dose (25 mg/kg, i.p.) was used to induce micronuclei in albino mice. Treatment with CTO was initiated in the single dose study (100, 300 & 500 mg/kg) and as a time course for 48 h and 72 h (500 mg/kg). 24 h after injecting cyclophosphamide, the animals were sacrificed and micronuclei were determined from smears prepared from bone marrow and peripheral blood. The antioxidant impact of the oil was determined using ferric ion reducing capacity of plasma and thiobarbituric acid reactive substances as a measure of lipid peroxidation. All doses were capable of preventing the formation of micronuclei but 500 mg/kg of the oil was most efficacious as a single dose and in the time course study. The beneficial effect of CTO is possibly due to the presence of antioxidants fractions such as Vit-E, linoleic acid and polyphenols.

**Keywords:** Cyclophosphamide, Carthamus tinctorius, Micronuclei, Linoleic acid, Vit-E.

## INTRODUCTION

Mutation is a heritable change in nucleotide sequence or number occurring due to alteration in the sequence of the code in a gene due to change, removal or insertion of one or more bases in a gene resulting in an altered gene product. In general, mutations are detrimental because they lead to defects in cellular functions. Large numbers of plant species are a great source of biologically active compounds whose effect on human health or genetic material is mostly unknown. The use of plant infusions to cure many different types of diseases is very common in Brazilian folk medicine. They frequently substitute for modern medicines. In recent years there has been greater interest in investigating compounds originating from plants and their effects on DNA. This is done with many different types of assays employing different organisms. The actions of these compounds may be involved in maintaining the balance between the consumption of mutagenic and antimutagenic substances, thus contributing to increases or reductions in the incidence of cancer in the population. Compounds from plants could act as protective agents with respect to human carcinogenesis, acting against the initiation, promotion or progression stages of this process [1]

or, perhaps, destroying or blocking the DNA-damaging mutagens outside the cells, thus avoiding cell mutations [2]. Mutagenic and antimutagenic activities have been correlated with the presence of certain phytochemical substances, such as compounds of the flavonoid group [1, 3]. A relationship has been reported between structure and activity, both for mutagenic activity and for protection of the genetic material (1, 4). Flavonoids are consumed naturally through the intake of beverages such as beer, coffee and wine (on average ~1 mg/l flavonoids) and may reach 25 mg/l in black tea. According to MacGregor and Jurd (1978) these are relatively stable compounds, resistant to heat, light and oxygen, and are moderately acidic [4].

Carthamus tinctorius oil (CTO) commonly referred to, as safflower oil is widely utilized edible oil. Seed oils are the main source of dietary ingredients related to their fatty acid composition and tocopherol content.Safflower has been grown since ancient times in semi-arid regions of the Middle East, northern Africa and India. It contains Myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid eicosenoic acid, behenic acid, lignoceric acid, tocopherol and polyphenols [5]. Carthamus tinctorius oil used for the treatment of hypertension, atherosclerosis, cystic fibrosis, fatty acid deficiency, menstrual disorder, traumatic injury, pain, friedreich's ataxia, skin problems, diabetes and used as an anticoagulant [6]. CTO is a rich source of tocopherol, linolenic acid,conjugated dienes, polyphenols and linoleic acid, which shows antioxidant activity. Till date, no work has been done on antimutagenic activity of CTO. Hence, this study reports the antimutagenic effect of Carthamus tinctorius oil on cyclophosphamide-induced mutagenicity in mice.

## MATERIALS AND METHODS

### Drugs

1. Cyclophosphamide- Bio chem. pharmaceutical Industries, India.
2. Carthamus tinctorius oil- Telidhar, Hyderabad.

### Animals

Healthy albino mice weighing 25-30 g were procured from National Institute of Nutrition, Hyderabad and acclimatized in our own animal house for one week prior to the experiment. The experimental animals were maintained in a temperature-controlled room (24 ± $1^0$C) with 12 h / 12 h light–dark illumination cycles and given water and food ad libitum. The institutional animal ethical committee has approved this experimental protocol and the experiments have been conducted as per guidelines.

### Experimental design

Albino mice divided in to VIII groups each group contains 6 animals each. Dose Dependent Study: Group I (negative control) animals treated with Treated with saline (0.1 ml), Group II (Positive control) animals Treated with Cyclophosphamide 25mg/kg b.w. Group III, IV and V animals were pretreated with CTO (100mg, 300 mg, 500mg /100g, b.w. orally) respectively and after 2 h administered Cyclophosphamide (25mg/kg b.w) by intraperitoneal route and after 24h sacrificed the mice.

Time Dependent Study Group VI animals pretreated with CTO (500 mg/100g b.w) once after 2 h the last administration of CTO, cyclophosphamide (25 mg/kg b.w) was administered by intraperitoneal route and animal was sacrificed after 24h. Group VI animals Pretreated with CTO (500 mg/100g b.w) daily once for 48 h after 2 h the last administration of CTO, cyclophosphamide (25mg/kg) was administered by intraperitoneal route and animal was sacrificed after 24h. Group VIII animals Pretreated with CTO (500 mg/100g b.w) daily once for 72 h after 2 h the last administration of CTO, cyclophosphamide (25mg/kg b.w) was administered by intraperitoneal route and animal was sacrificed after 24h. Blood was collected by retro orbital puncture under Enflurane anesthesia and centrifuged at 2380 g for 10 min to get plasma. Blood was divided into two portions one portion for preparing smear and the other for estimation of oxidative parameters.

### Estimation of total antioxidant capacity of CTO

CTO antioxidant status was evaluated using ferric reducing antioxidant power (FRAP) assay. The assay was carried out according to F.F.Benzie and J.J.Strain (1996). 100 µl of Carthamus tinctorius Oil was mixed with 3 ml of working FRAP reagent and absorbance was measured at 0

minute after vortexing at 593 nm. Thereafter, samples are placed at 37 °C in water bath and absorbance was again measured after 4 minutes. Ascorbic acid standards (100 µM-1000 µM) were processed in the same way. The total antioxidant capacity was determined using standard calibration curve of ascorbic acid and results were expressed in ascorbic acid equivalents as units of µmol/l. [7].

## Peripheral blood micronucleus assay

Then smears were made on clean glass slides. The slides were air-dried for 10 min. The slides were then fixed in absolute methanol. Then the slides were air-dried. These slides were kept for staining in coupling jars containing 1:5 mixture of Giemsa and phosphate buffer ($P^H$ 6.8) for 12-15min. Then the slides were washed with phosphate buffer ($P^H$ 6.8). These washed slides were kept in undisturbed phosphate buffer ($P^H$ 6.8) for 2-5 min for proper differentiation. Then the slides were air dried. Slides were observed under microscope. 3000 NCE were counted per group. [8]

## Total Antioxidant Capacity of Plasma

Plasma antioxidant status was evaluated using ferric reducing antioxidant power (FRAP) assay. The assay was carried out according to F.F.Benzie and J.J.Strain (1996). 100 µl of plasma was mixed with 3 ml of working FRAP reagent and absorbance was measured at 0 minute after vortexing at 593 nm. Thereafter, samples are placed at 37 °C in water bath and absorbance was again measured after 4 minutes. Ascorbic acid standards (100 µM-1000 µM) were processed in the same way. The total antioxidant capacity was determined using standard calibration curve of ascorbic acid and results were expressed in ascorbic acid equivalents as units of µmol/l. [7]

Estimation of Lipid Peroxidation in Plasma (Tbars Method)

The TBARs levels were estimated as per the spectrophotometric method described by Ohkawa etal.To each test tube, 0.5 ml of plasma and 0.5 ml of normal saline were added. To the above solution, 1 ml of 20% trichloroacetic acid (TCA) was added. To the above solution 0.25 ml of TBA reagent (200 mg of Thiobarbituricacid in 30 ml distilled water and 30 ml of acetic acid) was added. The test tubes were kept for boiling at 95ºC for one hour. To each of the test tubes, 3 ml of n-butanol was added and mixed well. The tubes were centrifuged at 856.8 g for 10 minutes. The separated butanol layer was collected. Blank was also prepared in the same way without plasma. The separated butanol layer was read in a spectrophotometer against reagent blank at 535 nm. The amount of lipid peroxidation was determined using the molar extinction coefficient of $1.56\times10^{-5}$ m/cm. Thiobarbituric reactive substances concentration was expressed in terms of µmol of malondialdehyde per millilitre of plasma [9].

## Bone Marrow Micronucleus Assay

The animals were sacrificed by cervical dislocation. Animals were dissected and opened to excise femur and tibia. Bones were then separated from muscle fibres by the use of gauze and fingers. Bone marrow MN slides were prepared by using the modified method of Schmid (1973), where 5% bovine serum albumin (BSA) was used as suspending medium for marrow cells (Seetha Rama Rao et al. 1983) instead of fetal calf serum. The bone marrow cells from femur and tibia were flushed with BSA. This cell suspension was centrifuged at 100.8 g for 7 min to isolate the bone marrow cell as a pellet. This pellet was resuspended in 1-2 drops of BSA. A drop of this suspension was taken on clean glass slides and smears were prepared. The slides were air-dried for 10 min. The slides were then fixed in absolute methanol. Air dried slides were stained with May-Grunewald (MG) and Giemsa as described by Schmid 1975. These slides were kept for staining in coupling jars containing 1:1 mixture of MG and phosphate buffer ($P^H$ 6.8) for 12-15min. These slides were transferred immediately to a coupling jar containing mixture of 1:5 Giemsa and phosphate buffer ($P^H$ 6.8) with the help of forceps and allowed to stand for 12 min. Then the slides were washed with phosphate buffer ($P^H$ 6.8). These washed slides were kept in undisturbed phosphate buffer ($P^H$ 6.8) for 2-5 min for proper differentiation. Then the slides were air-dried. MN was identified in two forms of red blood cells which are Polychromatic Erythrocytes as PCE and Normochromatic Erythrocytes as NCE using binocular microscope under oil immersion objective. About 3000 PCE and corresponding NCE per group was observed for the presence of MN [10, 11].

## STATISTICAL ANALYSIS

Data are expressed as mean ± SEM. Analysis of data was done by One-way ANOVA followed by Dunnett comparison test for FRAP and TBARS. Mann-Whitney U test was carried out for micronuclei in bone marrow and peripheral blood using Graph Pad In Stat version 3.10 for Windows 2009 (Graph Pad Software). The statistical significance was set as 0.05 (P<0.05).

## RESULT

### Total Antioxidant Capacity of CTO

Total antioxidant capacity of CTO is equivalent to 900 µmol/l of ascorbic acid.

### Peripheral Blood Micronucleus Assay

A single intraperitoneal exposure with cyclophosphamide (25 mg/kg) was capable of inducing significant increase in MN (MNNCE) within 24 h. Reduction in % MNNCEs was observed with 100 mg/100 g and 300 mg/100 g of CTO but 500 mg/100 g CTO exerted a significant reduction in the formation of MNNCEs induced by cyclophosphamide indicating that high dose of CTO is more effective in prevention of MN formation (P<0.05, Table-1 & Figure-1). In time dependent schedule, treatment with a dose of 500 mg/100 g of CTO for 72 h exerted a significant reduction in the formation of MNNCEs induced by cyclophosphamide, Compared with CP treated group (P<0.05, Table-2 & Figure-2 & 3).

**Table-1: Effect of CTO on the frequency of micronuclei in peripheral blood induced by Cyclophosphamide, dose dependent studies**

| Group | Treatment | Time/NCE | MNNCE | Percentage |
|---|---|---|---|---|
| G -I | Saline | 24 Hours / 3000 | 0.66±0.33 | 0.13±0.06 |
| G -II | Cyclophosphamide (CP) 25mg/kg | | 7.0±0.51 | 1.4±0.10 |
| G-III | CTO 100 mg/100 g + CP 25 mg/kg | | 5.0±0.73 | 1.0±0.14* |
| G-IV | CTO 300 mg/100 g + CP 25 mg/kg | | 4.0±0.51 | 0.8±0.10* |
| G-V | CTO 500 mg/100g + CP 25 mg/kg | | 3.0±0.36 | 0.6±0.07* |

Values are expressed as Mean ± SEM. N=6. Statistics: Mann-Whitney U Test: *P<0.05 compared with positive control, NCE: Normochromatic Erythrocytes. MNNCEs: Micronucleated Normochromatic Erythrocytes. CTO: Carthamus tinctorius oil. CP: Cyclophosphamide.

**Table - 2: Effect of CTO on the frequency of micronuclei in peripheral blood induced by cyclophosphamide, time dependent studies.**

| Group | Treatment | Time / NCE (3000) | MNNCE | Percentage |
|---|---|---|---|---|
| G-I | Saline | 24 h | 0.66±0.33 | 0.13±0.06 |
| G-II | Cyclophosphamide (CP) 25mg/kg | 24 h | 7.0±0.51 | 1.4±0.10 |
| G-VI | CTO 500 mg/100 g+ CP 25 mg/kg | 24 h | 3±0.51 | 0.60±0.10* |
| G-VII | CTO 500 mg/100 g + CP 25 mg/kg | 48 h | 2±0.57 | 0.4±0.11* |
| G-VIII | CTO 500 mg/100g + CP 25 mg/kg | 72 h | 1.0±0.51 | 0.1±0.10* |

Values are expressed as Mean ± SEM. N=6. Statistics: Mann-Whitney U Test: *P<0.05 compared with positive control, NCE: Normochromatic Erythrocytes. MNNCEs: micro nucleated Normochromatic Erythrocytes. CTO: Carthamus tinctorius oil. CP: Cyclophosphamide.

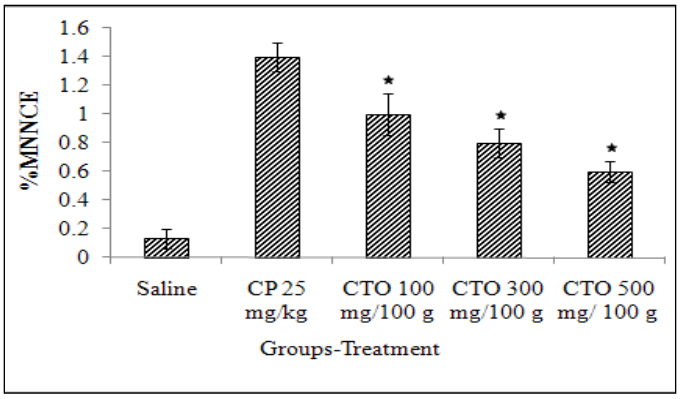



**Figure-1: Effect of CTO on the frequency of micronuclei in peripheral blood induced by cyclophosphamide, dose dependent studies.**

Values are expressed as Mean ± SEM. N=6. Statistics: Mann-Whitney U Test: *P<0.05 compared with positive control, NCE: Normochromatic Erythrocytes. MNNCEs: micro nucleated Normochromatic Erythrocytes. CTO: Carthamus tinctorius oil. CP: Cyclophosphamide.

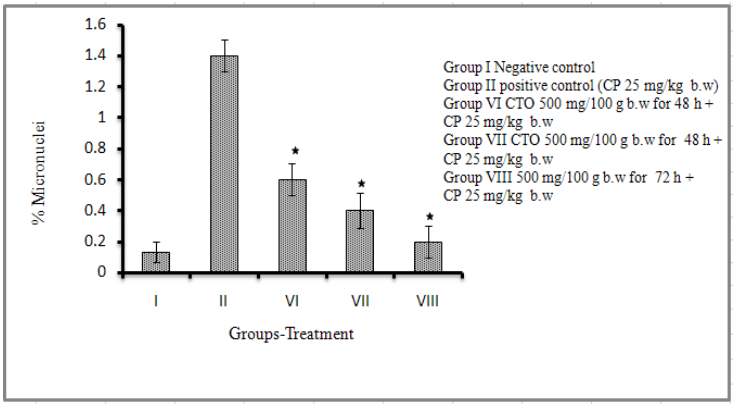



**Figure -2: Effect of COT on the frequency of micronuclei in peripheral blood induced by cyclophosphamide, time dependent studies.**

Values are expressed as Mean ± SEM. N=6. Statistics: Mann-Whitney U Test: *P<0.05 compared with positive control, NCE: Normochromatic Erythrocytes. MNNCEs: Micronucleated Normochromatic Erythrocytes. CTO: Carthamus tinctorius oil. CP: Cyclophosphamide.

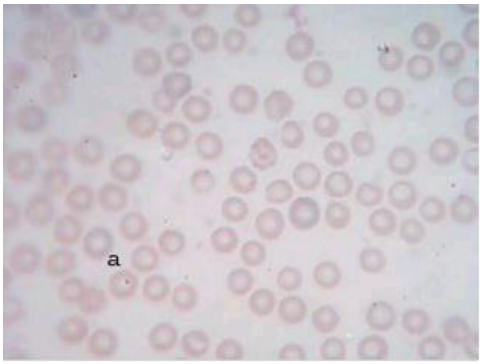



**Figure-3: Peripheral Blood Micronucleus Assay**

## Antioxidant Activity by Ferric Reducing Ability of Plasma (Frap) Assay

Treatment with CP in a dose of 25mg/kg b.w. exerted a decline in the FRAP value. Dose dependent increase in FRAP value was observed with CTO, however highest FRAP value was observed in group treated with 500 mg/ 100 g b.w. compared with CP treated group (P<0.05). Treatment with CTO for 72 h considerably increased the FRAP value to 800 µmol/l compared with CP treated group (P<0.05, Figure – 3&4).

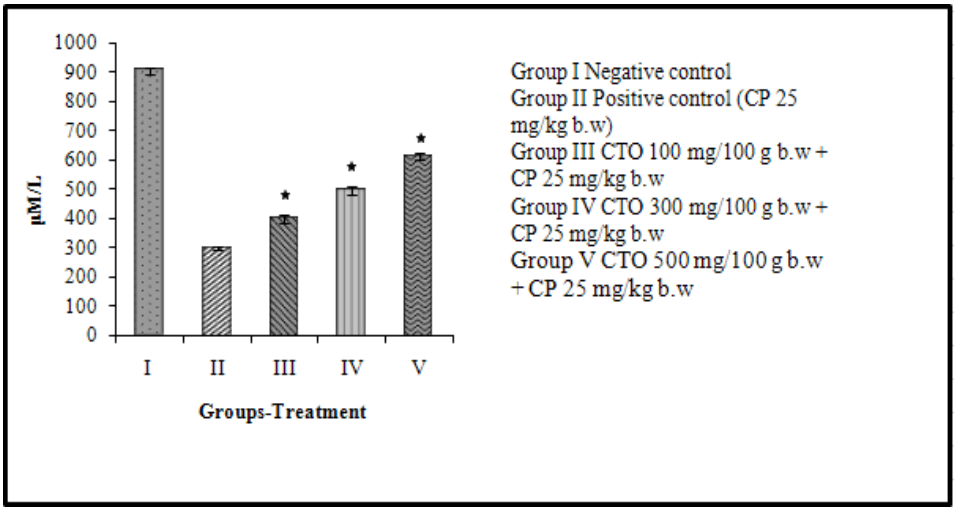



**Figure - 3: Effect of CTO on plasma antioxidant capacity in cyclophosphamide induced mutagenicity, dose dependent study**

Values are expressed as Mean ± SEM. N=6. Statistics: Dunnett Multiple Comparisons Test: *P<0.05 compared with positive control, CTO: Carthamus tinctorius oil. CP: Cyclophosphamide.

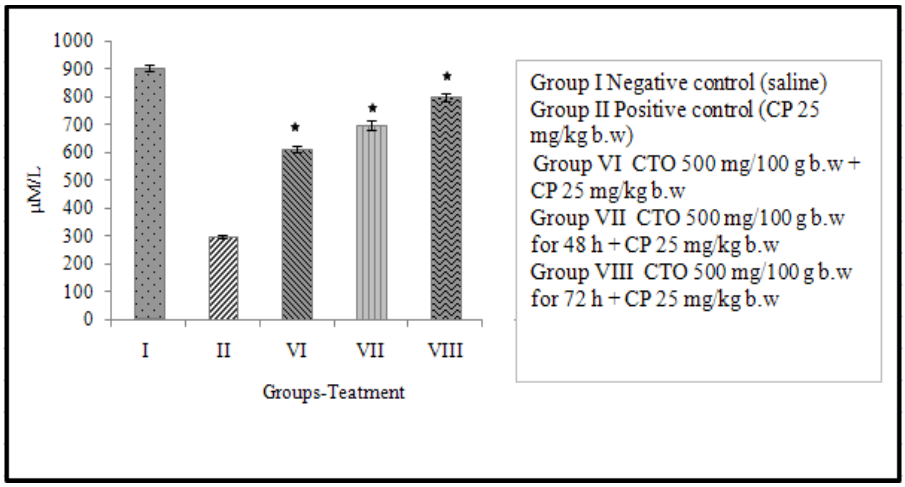



**Figure - 4: Effect of CTO on plasma antioxidant capacity in cyclophosphamide induced mutagenicity, time dependent study**

Values are expressed as Mean ± SEM. N=6. Statistics: Dunnett Multiple Comparisons Test: *P<0.05 compared with positive control, CTO: Carthamus tinctorius oil. CP: Cyclophosphamide.

## Estimation of thiobarbituric acid reactive substances (tbars) in plasma

Oxidative stress is represented by the concentration of TBARS. Group-5 treated with 500 mg/100 g should a decline in formation of lipid peroxides than animals treated with 100 mg/100 g

and 300 mg/100 g. When treatment was continued for 48 h and 72 h with 500 mg/100 g a significant reduction in the formation of oxidative stress markers was observed compared with CP treatment group (Table- 3 & 4, P<0.05).

**Table-3: Effect of CTO on formation of lipid peroxidation in plasma CP induced mutagenicity, dose dependent study.**

| Group | Treatment | Duration | TBARS (nmol/ml) |
|---|---|---|---|
| G -I | Saline | | 0.06±0.0011 |
| G -II | CP 25mg/kg | | 0.44±0.0057 |
| G -III | CTO 100mg/100g+CP25mg/kg | 24h | 0.38±0.0057* |
| G -IV | CTO 300mg/100 g+CP25mg/kg | | 0.34±0.006* |
| G -V | CTO 500mg/100g+CP 25mg/kg | | 0.3±0.009* |

Values are expressed as Mean ± SEM. N=6. Statistics: Dunnett Multiple Comparisons Test: *P<0.05 compared with positive control, CTO: Carthamus tinctorius oil. CP: Cyclophosphamide.

**Table 4: Effect of CTO on formation of lipid peroxidation in plasma CP induced mutagenicity, time dependent study**

| Group | Treatment | Duration | TBARS (nmol/ml) |
|---|---|---|---|
| G -I | Saline | 24 h | 0.06±0.0011 |
| G -II | CP 25mg/kg | 24 h | 0.44±0.0057 |
| G -VI | CTO 500 mg/100 g+CP25 mg/kg | 24 h | 0.3±0.009* |
| G -VII | CTO 500 mg/100g+CP25 mg/kg | 48 h | 0.25±0.00097* |
| G -VIII | CTO 500 mg/100g+CP25 mg/kg | 72 h | 0.11±0.003* |

Values are expressed as Mean ± SEM. N=6. Statistics: Dunnett Multiple Comparisons Test: *P<0.05 compared with positive control, CTO: Carthamus tinctorius oil. CP: Cyclophosphamide.

## BONE MARROW MICRONUCLEUS ASSAY

In dose-dependent study, a reduction in the frequency of MNPCEs and MNNCEs were observed with100 mg/100 gm and 300 mg/100 gm treated group, implying its ability to affect the formation of MN. Treatment with 500 mg/100 gm of CTO exhibited a fair reduction in MNPCEs and MNNCEs formed compared with the cyclophosphamide treated group (P<0.05). The proportion of MNPCEs and MNNCEs formed declined as the duration of treatment with CTO (500 mg/100 gm) progressed (Figure 5, 6 & 7 P<0.05).

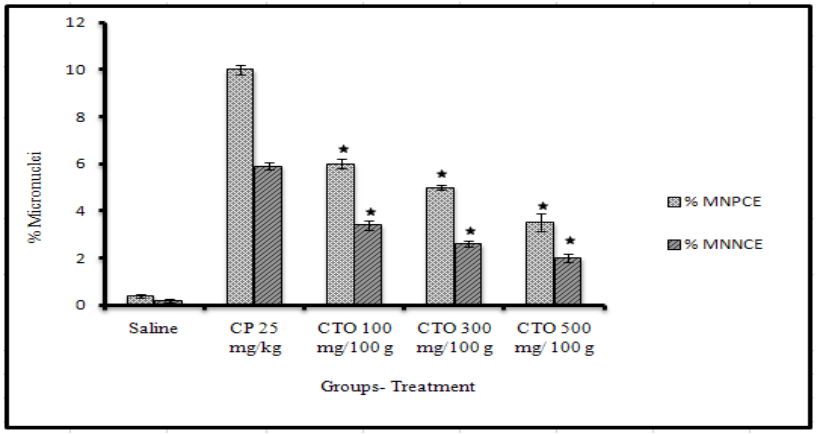



**Figure 5: Effect of CTO on the frequency of micronuclei in bone marrow induced by cyclophosphamide, dose dependent studies.**

Values are expressed as Mean ± SEM. N=6. Statistics: Mann-Whitney U Test: *P<0.05 compared with positive control, CP.

MNPCEs: Micro nucleated Polychromatic Erythrocytes

MNNCEs: Micro nucleated Normo chromatic erythrocytes.

CTO: Carthamus tinctorius oil.

CP: Cyclophosphamide

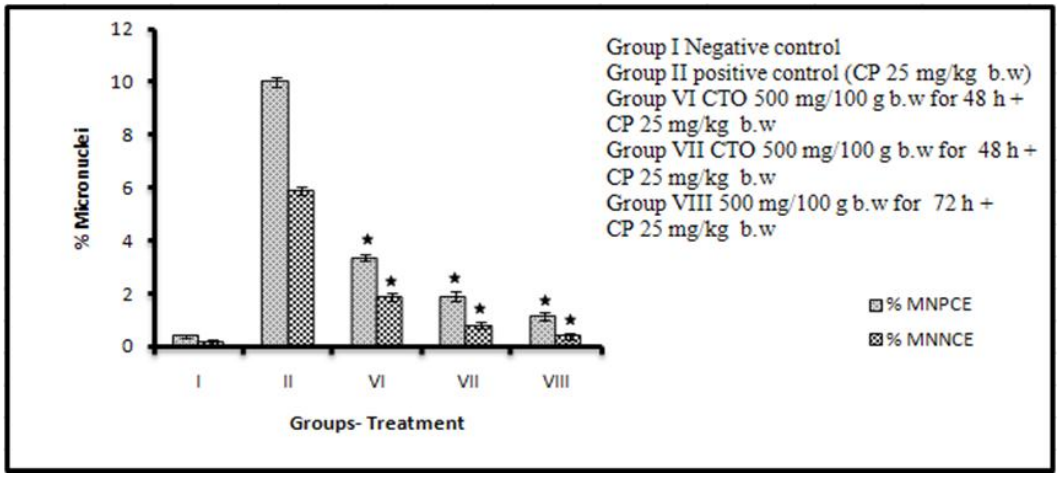
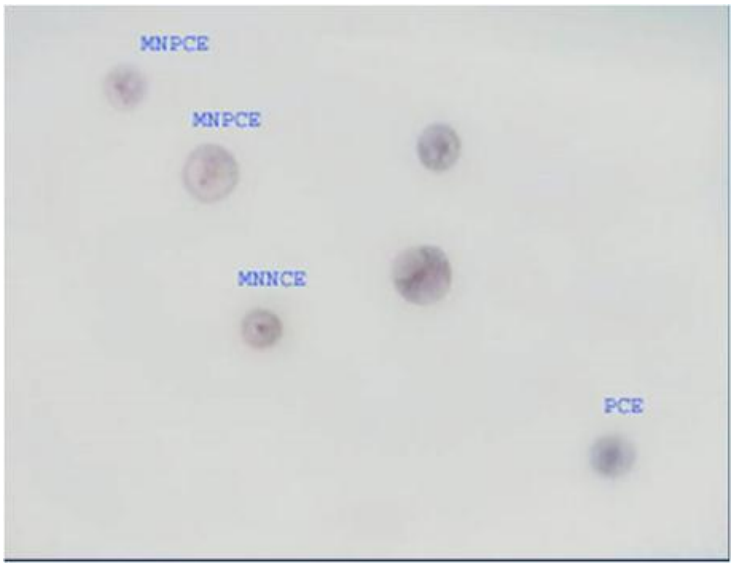
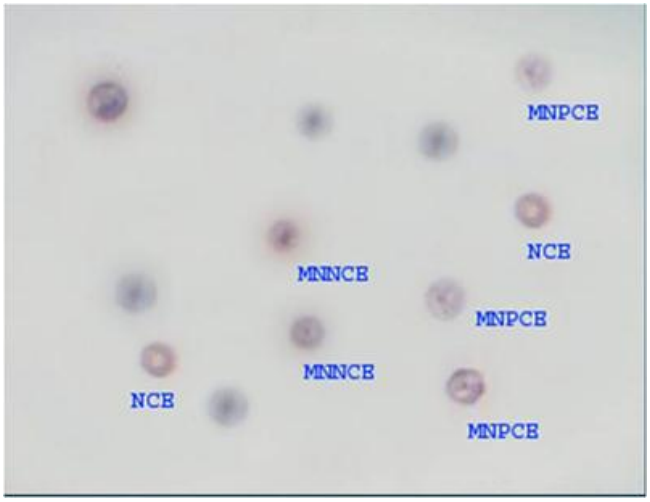



**Figure 6: Effect of Carthamus tinctorius oil on the frequency of micronuclei in bone marrow induced by cyclophosphamide, time dependent studies.**

Values are expressed as Mean ± SEM. N=6. Statistics: Mann-Whitney U Test: *P<0.05 compared with positive control, CP. PCE: Polychromatic erythrocytes. MNPCEs: micro nucleated Polychromatic Erythrocytes. NCE: Normochromatic Erythrocytes MNNCEs: Micro nucleated Normochromaticerythrocytes. CTO: Carthamus tinctorius oil.CP: Cyclophosphamide.






## DISCUSSION

The present study was designed to investigate the ability of Carthamus tinctorius oil to attenuate CP induced mutagenicity and oxidative stress in peripheral blood and bone marrow. CP is one of the most widely advocated cytostatic agents with tremendous potential of curbing uncontrolled proliferation of cells. It is particularly beneficial as a immunosuppressant in preventing organ rejection after transplants and in the mitigation of autoimmune diseases. Biologically, cyclophosphamide can be regarded as a prodrug requiring metabolic activation by CYP-P-450 mixed function oxidases converting it into a therapeutically beneficial cytotoxic product. Due to formation of phospharamide mustard, alkylation of DNA occur leading to its damage and destruction. Acrolein is a toxic by product produced along with phospharamide mustard, which is capable of inducing potential oxidative stress and DNA damage. Due to this CP is believed to tilt the redox balance, enhance lipid peroxidation and limit the activity of anti-oxidant enzymes and is considered as good inducer of genotoxicity.

The most versatile cytogenetic assay to assess DNA damage is the micronucleus assay developed by Schmid. The test enables one to clearly demarcate between a mutagen and antimutagen. Newly formed erythrocytes referred to as polychromatic erythrocytes and mature forms called normochromatic erythrocytes are derived from the stem cells in the bone marrow and are considered for MN assessment in the erythropoietic cascade. Formation of MN in PCE and NCE on exposure to xenobiotics serve as a important index of genotoxic propensity. A definite elevation in the frequency of MNPCEs and MNNCEs in bone marrow and peripheral blood was observed in our studies following exposure to CP. The incline in the formation of TBARS, an important marker of lipid peroxidation and decline in FRAP value imply that CP can cause DNA damage through oxidative stress. The liberation of free radicals during the process can insult DNA leading to mutation. This could further produce DNA strand breaks, base modification, chromosomal aberration finally culminating in the formation of MN.

Treatment with CTO has shown a dose dependent decline in the number of MNPCEs and MNNCEs in bone marrow and peripheral blood. Evaluation of antioxidant status has shown an appreciable decrease in the formation lipid peroxides and enhanced FRAP value following treatment with CTO. DNA in cell is susceptibles to 100000 oxidative lesions per day. CP can produce a shift in the oxidant /antioxidant balance out numbering the oxidative lesions in DNA. CP is advocated for several conditions, however the risk involved its use manifold. Production of the internal machinery of cell and genomic stability could be ensured by incorporating nutritional supplement in the diet by fortification of foods. Seed oils are unique constituents due to their fatty acid and tocopherol content. Vitamins are important as their function as co factor in maintenance of stable DNA. CTO contains PUFAs and is rich in natural antioxidants like Vit-E which minimize enhance of oxidative stress. Protection of DNA cold also be elicited by CTO due to the presence of linoleic acid (LA). The antioxidant

response element (ARE) is a cis-acting enhance element which could be activated by LA. Due to its activation expression of several anti-oxidant enzymes can take place. Thereby reducing the impact of free radicals on DNA.

Our findings reveal that treatment with CTO for 72 h has diminished the formation of MNPCEs and MNNCEs in the micronucleus assay carried out using bone marrow and peripheral blood.

## SUMMARY AND CONCLUSION

Mutation is a heritable change in nucleotide sequence or number occurring due to alteration in the sequence of the code in a gene due to change, removal or insertion of one or more bases in a gene resulting in an altered gene product. Mutations exert detrimental effects by causing defects in cellular functions. The tremendous rise in the number of industries and automobiles has resulted in the generation of several harmful chemicals. Human beings are constantly exposed to these chemicals through food, water and environment. Hazards induced by mutagens are at the molecular level causing DNA damage. Mutagens induce mutations through ROS formed during the metabolic processes of highly reactive mutagens which can attack DNA bases to produce oxidized bases or single and double strand breaks. Cyclophosphamide (CP) and its metabolites can bind DNA, causing damage that may result in chromosome breaks and micronucleus formation. Micronuclei are small spherical chromatin elements which lie outside the nucleus in a cell or in erythrocytes. They appear identical in structure, shape and size to the main nucleus in a cell. Exposure to CP can contribute significantly to oxidative stress leading to the generation of free radicals. Dietary constituents which are rich in antioxidants might be capable of partially or completely inactivating mutagens or facilitating DNA repair, keeping this in mind the present study was undertaken. Seed oil like Carthamus tinctorius which is the major source of fatty acids and tocopherol has been explored in this study. The results reveal that high dose of safflower oil shows better action against cyclophosphamide induced mutagenicity thereby reducing oxidative stress. The anti-mutagenic effect of safflower oil was due to presence of Vit-E, polyphenols, linoleic acid.

Hence, it can be concluded that CTO is effective in combating the deleterious effects induced by cyclophosphamide because of its rich antioxidant property. It was observed that total antioxidant capacity of Carthamus tinctorius oil was equivalent to 900 µM/L of ascorbic acid. From the present study, it was concluded that CTO was effective at dose of 500 mg/100 g in dose dependent study. In time course study, CTO was effective at dose of 500 mg/100 g when treated for 72 h in FRAP indicating the effective antioxidant activity. The present study indicates that CTO was effective at dose of 500 mg/100 g in dose dependent study, in time course study, CTO was effective at dose of 500 mg/100 g when treated for 72 h in peripheral blood and bone marrow micronucleus assays. A reduction in the formation of an important marker of oxidative stress which is TBARS was observed following treatment with CTO in a dose of 500 mg/100 g. CTO in high dose as a single schedule for 72 h was found to be effective in reducing oxidative stress.

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