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Research Paper

EVALUATION OF GENOTOXIC AND ANTIGENOTOXIC POTENTIAL OF MEDICINAL PLANTS (GARCINIA INDICA AND HIBISCUS ROSA SINENSIS) IN SWISS ALBINO MICE

Kumar Ajay* and Kaushal K. Chandrul

Shri Venkateshwara University, Gajraula, Uttar Pradesh. India

Genotoxic profile of a drug can be established by *in vitro* and *in vivo* tests designed to detect genetic damage directly or indirectly by various mechanisms. Environmental and chemical mutagenic drugs disturbs DNA synthesis and cell division, they may have damaging effect on tissues with normally low mitotic indices. They have Cytotoxic and radiomimetic actions generating reactive oxygen species such as Superoxide (O_2^-); Nitric oxide (NO), Hydrogen peroxide (H_2O_2) and Peroxy radicals in a biological system. These reactive oxygen species have been implicated in certain chronic and ageing diseases, including malaria, rheumatoid arthritis, cataracts acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes, cancer and neurodegenerative diseases (Parkinson's and Alzheimer's diseases).

Garcinia indica has been reported to possess cytotoxic activity, ascribed to its chemical constituent, xanthochymol and isoxanthochymol and its antioxidant activity is attributed to garcinol, hydroxycitric acid and other chemical constituents. By virtue of its cytotoxic activity the plant could be expected to possess genotoxic activity and due to its antioxidant property it could prevent Genotoxicity of anticancer drug, when administered with the latter. Due to paucity of information about the plant in this regard, the present study was planned to evaluate aqueous fruit rind extract of *garcinia indica* for its genotoxicity if any, and its effect on cyclophosphamide induced genotoxicity.

Hibiscus rosa sinensis linn (fam: malvaceae) is one such Indian herbal plant used extensively to treat a spectrum of ailments. *Hibiscus rosa sinensis* has been reported in prevention of two-stage skin carcinogenesis. As per latest information no invivo antigenotoxic or anticlastogenic activity of this drug has been performed, therefore the present study was designed to investigate in vitro antioxidant and genotoxic potential if any and effect on cyclophosphamide induced genotoxity in mice, using micronucleus assay and COMMET assay as experimental models. Genotoxicity can be detected by a variety of tests like Ames test, micronucleus test, chromosomal aberration test, comet assay (SCGE), unscheduled DNA synthesis assay, point mutation assay etc. Comet assay is simple, sensitive and rapid method for screening DNA double and single strand breaks, which can be further confirmed by micronucleus assay.

KEY WORDS: Genotoxicity, Clastogenicity, Aneugenicity, Anticlastogenic activity, Genetic toxicology, Mutations, Micronucleus assay and Carcinogenesis.

INTRODUCTION

Genetic toxicology is defined as the study of adverse effects on the process of heredity. It has been given the task of detecting mutagenic chemicals using an array of tests. Genotoxic profile of a drug can be established by *in vitro* and *in vivo* tests designed to detect genetic damage directly or indirectly by various mechanisms. Cytogenic markers such as chromosome aberrations (abnormality in chromosomal structure and number), micronuclei frequency and sister chromatid exchanges are relatively rapid, facile and

sensitive indicators of genetic damage.[1-5]

Genotoxic agents such as carcinogens can enhance the error rate in the genome reduplication and cause mutation in the DNA of an organism. Cancer patients are often exposed to high level of DNA damaging agents. [6]

Environmental and chemical mutagenic drugs have Cytotoxic and radiomimetic actions generating reactive oxygen species such as Superoxide (O_2^-); Nitric oxide (NO), Hydrogen peroxide (H_2O_2) and Peroxy radicals in a biological system.[7]

Mammalian cells possess elaborate defence mechanisms for free radical detoxification. Key metabolic enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) and high and low molecular weight compounds e.g. albumin, tocopherol, minerals like selenium, copper and vitamins such as Vit. A and Vit. C etc. play a crucial role in preventing oxidative stress in the biological system[8].

Antioxidants are compounds that help to inhibit the many oxidation reactions caused by free radicals, thereby preventing or delaying damage to the cells and tissues. Therefore, the commercial development of plants as sources of antioxidants to enhance health and food preservation is of current interest. [9-11]

Garcinia indica has been reported to possess cytotoxic activity, ascribed to its chemical constituent, xanthochymol and isoxanthochymol and its antioxidant activity is attributed to garcinol, hydroxycitric acid and other chemical constituents. By virtue of its cytotoxic activity the plant could be expected to possess genotoxic activity and due to its antioxidant property it could prevent Genotoxicity of anticancer drug, when administered with the latter. Due to paucity of information about the plant in this regard, the present study was planned to evaluate aqueous fruit rind extract of *garcinia indica* for its genotoxicity if any, and its effect on cyclophosphamide induced genotoxicity. [12-13]

Toxicology and Genetic Toxicology:

Toxicology is defined as "the study of the adverse effects of chemical, physical or biological agents on living organisms and the ecosystem". [14]

Routine, assessment of toxicity in experimental studies is done by physical observations or biochemical investigations. The adverse events of drugs such as genetic mutation may go unnoticed until it manifests as diseased condition including malignancy. Drugs like cyclophosphamide used in malignancy are well established genotoxic agents. [15]

The simplest functional unit in a DNA molecule is termed as gene and they are ultimate units of heredity. Their normal expression and regulation is essential for all physiological functions and their abnormality/deficiency forms the basis for many clinical conditions. The most common cause for gene malfunction is DNA damage. The term damage applies not only to mutations resulting from exposure to exogenous agents

like UV, radiation and heat, but also to 'spontaneous' mutations due to DNA lesions that originate endogenously. Various DNA damages are shown in **fig (2) and fig (3)**. A significant fraction of DNA damage involves the modification or elimination of bases, without alteration of the sugar–phosphate backbone, so that DNA coding capacity is greatly reduced or even abolished at the lesion site .[16]

Garcinia indica *Synonym: -**Garcinia purpurea Roxb***. Family-**Clusiaceae,** consist of dried fruits rind known as kokum, bark, and young leaves. It is distributed in, Western Ghats, most commonly in the Southern Konkan and Goa. It is an Indian spice used for making several vegetarian and non-vegetarian 'curry' preparations, including the popular 'solkadhi'. The fruits are steeped in sugar syrup to make 'amrut kokum', a healthy soft drink to relieve sunstroke, which is popular during summer.[17]

Fig. 1: Photograph showing plant of *Garcinia indica*

Garcinia indica C is distributed mainly in peninsular India. This is one of several species of Garcinia found in many tropical regions. The Kokum is from a tall tropical evergreen tree. The fruit is harvested during April-May of every year. The extract of the fruit has both antifungal and antibacterial properties and therefore, has a potential for use as biopreservative in food applications. It is traditionally used to treat sores, skin ailments such as rashes caused by allergies, dermatitis and chaffed skin, burns, scalds, and to relieve sunstroke. It is also a remedy for diarrhea, dysentery, piles and tumors. It facilitates digestion, purifies the blood and fights cholesterol.

It has been found that rind of the fruit contains hydroxy citric acid [HCA], garcinol and the coloring pigment anthocyanin. HCA, which is claimed to have fat-reducing properties, is often used to reduce obesity, since it inhibits the enzyme, citrate lyase responsible for conversion of carbohydrates into fats. Another major compound reported to be present in the chloroform extract of the fruit is garcinol with a strong antioxidant activity since it contains both phenolic hydroxyl groups as well as a β-diketone moiety and it exerts an anti-inflammatory effect. It acts as a free radical scavenger and hence is very important pharmaceutically. This study focuses to characterize the active compounds responsible for antibacterial activity of *Garcinia indica* against strains of bacteria, which cause digestive tract disorders and mild skin infections.[18]

Scientific Classification :

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SCIENTIFIC CLASSIFICATION: *GARCINIA INDICA*	
Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Malpighiales
Family:	Clusiaceae
Subfamily:	Clusioideae
Tribe:	Garcinieae
Genus:	*Garcinia*
Species:	*G. indica* Chois.

The plant is known by various names in different languages as follows:

Sanskrit	Atya amla , Amlavesta
Hindi	Kokum
Marathi	Kokamb , Amsol
Kannada	Murgal
Tamil	Murgal mara
Gujrati	Kokan

Hibiscus rosa-sinensis[19], known colloquially as **Chinese hibiscus**, **China rose**, **Hawaiian hibiscus**, **rose mallow** and **shoeblackplant**, is a species of tropical hibiscus, a flowering plant in the Hibisceae tribe of the family Malvaceae. It is widely cultivated in tropical and subtropical regions, but is not known in the wild, so that its native distribution is uncertain. An origin in some part of tropical Asia is likely. ***nomancleature:*** *Hibiscus rosa-sinensis* was named in 1753 by Carl Linnaeus in his *Species Plantarum*. The Latin term *rosa-sinensis* literally means "rose of China", though it is not closely related to the true roses. *Hibiscus rosa-sinensis* is a bushy, evergreen shrub or small tree growing 2.5–5 m (8–16 ft) tall and 1.5–3 m (5–10 ft) wide, with brilliant red flowers in summer and autumn. The 5-petaled flowers are 10 cm (4 in) in diameter, with prominent orange-tipped red anthers. Flower color in certain species, such as *H. mutabilis* and *H. tiliaceus*, changes with age This flower has a crown that consists of 15 - 20 leaves which are

Fig. 2: Pollen parent

Fig. 3: Pod parent

Fig. 4: offspring

Fig. 5: Another offspring

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alternate and ovate.

Fig. 6: China Rose

At the bottom of every hibiscus bud is the calyx which is green in color. When the hibiscus begins to bloom, the petals begin to grow which contains multiple petals and multiple colors. The ovary and other female parts of the flower lie in the main structure of the hibiscus, the pistil, which is long and tubular. The hibiscus has both male and female parts on the same flower. The five hairy red spots on the top of the flower is the stigma (female part) of the flower. The stigma is located at the end of the style branch. The ovary lies at the bottom of the blossom and the hibiscus has only one ovary which is superior.

The male part (stamen) of the flower consists of stem-like filaments and each filament ends with the pollen-producing anther. Overall, the hibiscus is a dicot, solitary (axillary), complete, perfect, has a superior ovary, regular symmetry, and axile placentation. It has 5 carpels, 5 locules, 5 sepals, and the amount of stamens may vary.

The root is a branched tap root. The stem is aerial, erect, green, cylindrical and branched.

The leaf is simple, with alternate *phyllotaxy* and is petiolate. *Venation* is unicostate reticulate.

Materials and Methods [20-21]

Collection and Authentification: The Fruit of

SCIENTIFIC CLASSIFICATION: *HIBISCUS ROSA-SINENSIS*	
Kingdom:	**Plantae**
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Malvales
Family:	Malvaceae
Genus:	*Hibiscus*
Species:	*H. Rosa-Sinensis*
Binomial Name	*Hibiscus Rosa-Sinensi* L.

Garcinia indica and *Hibiscus rosa-sinensis* was collected from the Goa region**.** The fruit was authenticated by Dr. Harsha Hegde, Research officer, Regional Medical Research Centre, Nehru Nagar, Belgaum.

Preparation of Aqueous Extract: About 50 gm of dried fruit rind powder was macerated with distilled water for seven days to get aqueous extract and concentrated using rotary evaporator and dried in desiccator. Then the extract was weighed and percentage extractive value was determined. The percentage yield was 47.68% w/w and extract were kept in refrigerator for further use.

Qualitative Chemical Investigation of Extracts: A qualitative chemical test was

conducted for the *Garcinia indica* fruit rind extract to identify the various phytoconstituents, given in the **Table no.5.**

1. Tests for Carbohydrates:

Molish's test (General test): To 2-3 ml aqueous extract, added few drops of α-naphthol solution in alcohol, shaken and added conc. H_2SO_4 from sides of the test tube was observed for violet ring at the junction of two liquids.

For Reducing Sugars:-

Fehling's test: 1 ml Fehling's A and 1ml Fehling's B solutions was mixed and boiled for one minute. Added equal volume of test solution. Heated in boiling water bath for 5-10 min was observed for a yellow, then brick red precipitate.

a) **Benedict's test**: Equal volume of Benedict's reagent and test solution in test tube were mixed. Heated in boiling water bath for 5 min. Solution may appear green, yellow or red depending on amount of reducing sugar present in test solution.

Tests for Monosaccharide:

***Barfoed's test*:** Equal volume of Barfoed's reagent and test solution were added. Heated for 1-2 min, in boiling water bath and cooled. Observed for red precipitate.

Tests for Hexose Sugars:

Cobalt-chloride test: 3 ml of test solution was mixed with 2ml cobalt chloride, boiled and cooled. Added $FeCl_3$ drops on NaOH solution. Solution observed for greenish blue (glucose), purplish (Fructose) or upper layer greenish blue and lower layer purplish (Mixture of glucose and fructose).

Tests for Non-Reducing Sugars:

a) Test solution does not give response to Fehling's and Benedict's test.

Test for Non-Reducing Polysaccharides (Starch):

a) Iodine test: mix 3 ml. test solution and few drops of dilute Iodine solution. Blue colour appears; it disappears on boiling and reappears on cooling.

b) ***Tannic acid test for starch:*** With 20% tannic acid, test solution was observed for precipitate.

2. Tests for Proteins:

a) ***Biuret test (General test):*** To 3 ml T.S added 4% NaOH and few drops of 1% C_4SO_4 solution observed for violet or pink colour.

b) ***Million's test (for proteins):*** Mixed 3 ml T.S. with 5 ml Million's reagent, white precipitate. Precipitate warmed turns brick red or precipitate dissolves giving red colour was observed.

c) ***Xanthoprotein test*** (For protein containing tyrosine or tryptophan): Mixed 3ml T.S. with 1 ml concentrated H_2SO_4 observed for white precipitate.

d) ***Test for protein containing sulphurs:*** Mixed 5 ml T.S. with 2 ml 40% NaOH and 2 drops 10% lead acetate solution. Solution was boiled it turned black or brownish due to Pbs formation was observed.

4. Tests for Steroid:

a) ***Salkowski Reaction:*** to 2 ml of extract, 2 ml chloroform and 2 ml conc. H_2SO_4 was added. Shaked well, whether chloroform layer appeared red and acid layer showed greenish yellow fluorescence was observed.

b) ***Liebermann-Burchard Reaction:*** Mixed 2ml extract with chloroform. Added 1-2 ml acetic anhydride and 2 drops conc. H_2SO_4 from the side of test tube observed for first red, the blue and finally green colour.

c) ***Libermann's reaction:*** Mixed 3 ml extract with 3 ml acetic anhydride. Heated and cooled. Added few drops concentrated H_2SO_4 observed for blue colour.

5. Tests for Flavonoids:

a) ***Shinoda test:*** To dried powder or extract, added 5 ml 95% ethanol, few drops concentrated HCl and 0.5 g magnesium turnings. Pink colour was observed.

b) ***Ferric Chloride test:*** Test solution with few drops of ferric chloride solution shows intense green colour.

c) ***Alkaline reagent test:*** Test solution when treated with sodium hydroxide solution shows increase in the intensity of yellow colour which becomes colourless on addition of few drops of dilute acid.

d) ***Lead Acetate solution test:*** Test solution with few drops of lead acetate solution (10%) gives yellow precipitate.

IN-VITRO ANTIOXIDANT ACTIVITY[22]:

- DPPH (2, 2-diphenyl-2-picryl hydrazyl) radical scavenging activity
- ABTS(2,2-azinobis(3-ethylbenzothiazoline–6–sulfonicacid)radical decolourization assay
- NITRIC OXIDE scavenging assay
- INHIBITION OF LIPID PEROXIDATION

Methodology[23]

Radical Generation and Scavenging Studies: A stock solution of 1 mg/ml of GI was prepared in Double distilled water and diluted to get various concentrations (100–1000 µg/ml) in a final volume of the reaction mixture. The free-radical scavenging activity of the extract was analysed by following the various standard in vitro radical generating model systems viz., 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azinobis (3-ethylbenzothiazoline–6–sulfonic acid), (ABTS) and Nitric oxide (NO). In all the experiments, double distilled water served as blank and reaction mixtures without GI served as control samples. The changes in the absorbance of the reaction mixtures were measured using a Spectrophotometer and the percent scavenging or inhibition was calculated according to the following formula.

$$\text{Percent scavenging or inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

DPPH scavenging activity of GI **in vitro:** The principle of the reduction of DPPH free-radical assay is that antioxidants react with the stable DPPH radical and convert it into 1, 1-diphenyl-2-

picryl hydrazine. The ability to scavenge the stable DPPH radical is measured by a decrease in the Absorbance. Aliquots containing various Concentrations (100–1000µg /ml) of GI in the final volume of 2 ml were mixed with 2 ml of 0.05 mM DPPH (in methanol). DPPH (2 ml) without GI served as control. Reaction mixtures were incubated at 37^{O}C for 20 min and the absorbance of reaction mixtures was recorded at 517 nm.

ABTS radical decolourization assay: The principle of the ABTS (2,2-azinobis (3- ethyl benzothiazoline –6– sulfonic acid) diammonium salt cation radical decolourization assay is that the antioxidants react with ABTS resulting in the decolourization of the ABTS radical in aqueous phase. The stock solution of ABTS was prepared by dissolving 4mg of it in 10ml.Double distilled water, then 38mg of Pot.Persulphate was taken and dissolved in 1ml. double distilled water, from this 88µl of Potassium persulfate ($K_2S_2O_8$) was added to 10 ml of ABTS solution, and kept for overnight incubation at 4^{O}C. The working ABTS reagent was prepared by diluting the stock solution with ethanol to give an absorbance of 0.7 ± 0.5 at 734 nm. Various concentrations of GI (100–1000 µg/ml) in a final volume of 1 ml were mixed with 1 ml of the ABTS cation working solution. Absorbance of reaction mixtures was recorded at 734 nm. In all the experiments, double distilled water served as blank and reaction mixtures without GI (1ml of working solution) served as control samples The percent scavenging or inhibition was calculated.

Scavenging of Nitric oxide by GI: The reaction mixture contained 1 ml of Sodium nitroprusside (50mM) in PBS containing 150µl of various GI concentrations (100–1000µg/ ml) along with the control (Sodium nitroprusside without GI) were incubated at room temperature (28^{O}C) for 3-4 hrs. After incubation 0.5ml of reaction mixture was discarded from each test tube followed by the addition of 1 ml of Greiss reagent (prepared by mixing an equal volume of 1% Sulphanilamide in 2% Phosphoric acid with 0.1 % N- (Naphthyl) ethylene diamine hydrochloride in water). The absorbance of the chromophores formed during diazotization of nitrate with Sulfanilamide and the subsequent coupling with N- (1-Napthyl) ethyenediamide was read at 546 nm. The changes in the absorbance of the reaction mixtures were measured using a spectrophotometer and the percent scavenging or inhibition was calculated.

Inhibition of Lipid Peroxidation by GI .: Approximately 10% (w/v) mouse brain homogenate was prepared in normal saline, and was stored at freezing conditions. The reaction mixture contained 0.3 ml of 20% brain homogenate, 0.3 ml of 150 mM Potassium chloride and 500µl of various concentrations of GI (100–1000µg/ml). The reaction mixtures without GI served as control. Peroxidation was initiated by adding 0.1 ml of 20 mM Ferrous sulfate ($FeSO_4$) .After incubating the mixture for

20 min at 37^{O}C (pulsating water bath). The reaction was stopped by the addition of 2ml of 0.25N HCl containing 15%TCA, 0.38%TBA and 0.05%BHT and then heated in a water bath at 65^{o}C for 30min. The reaction mixtures were cooled and centrifuged at 1500 r.p.m. for 10 min. Absorbance of the TBARS in the supernatant was recorded at 532 nm. The changes in the absorbance of the reaction mixtures were measured using a Spectrophotometer and the percent scavenging or inhibition was calculated.

Toxicity Studies[24]

Acute Oral toxicity – Acute Toxic Class method: The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Cooperation and Development (OECD), received draft guidelines 423, received from Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

DESCRIPTION OF THE METHOD

1) Selection of animal species: Healthy young albino mice of either sex weighing between 25 to 40g were used for acute toxicity study to determine LD_{50} of various extracts.

2) Housing and feeding condition: The temperature in the experimental room was around 25^{0}C. Lightning sequence was of 12 hours dark, 12 hours light. The conventional laboratory diet was fed, with an unlimited supply drinking water.

3) Preparation of animals: The animals were randomly selected, marked to permit individual identification, and kept in their cages for five days prior to dosing to allow for acclimatisation to the laboratory condition.

4) Preparation of doses: All the extracts were prepared as a suspension by triturating with distilled water.

5) Administration of doses: The test substances were administered in a single dose by gavage using a stomach tube. Animals were fasted prior to dosing, following the period of fasting, the animals were weighed and test substance was administered. After the dose was administered, food was withheld for a further 3-4 hrs.

6) Number of animals and dose levels: In each steps six animals were used in each group. Starting dose was 300 mg / kg body weight up to 2000 mg / kg body weight. The procedure of dose selection and finalizing LD_{50} cut off values is shown in the Table No. 1

Table 1: Dose Selection and Finalizing LD_{50} Cut Off value of Aqueous Extract.

S.No	Test Drugs	Mice dose(mg/kg)
1.	Cyclophosphamide	40mg/kg
2.	Vitamin e	50 iu/kg
3.	Gi extract	200mg/kg

1/10th of this lethal dose was taken as effective dose (therapeutic dose) for subsequent Genotoxic and antigenotoxic activity.

Table 2: Calculated doses of drugs used in the present study

S. No	Name of Extract	LD_{50} Cut-Off mg/ kg, b.w	Vehicle
1.	Aqueous extract	2000 mg/kg, b.w.	Water

7) Observations: Animals were observed initially after dosing at least once during the first 30 minutes, periodically during the first 24 hours. In all cases death was observed within first 24 hours. Additional observations like changes in skin and fur, eyes and mucous membranes was also noticed.

GENOTOXICITY STUDIES[25]

Healthy Albino mice (*Mus musculus*) weighing 20-25 g of either sex were used for the study. The experimental protocol was approved by Institutional Animal Ethics Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA) at J.N.Medical College Belgaum, India.

Swiss albino mice were fed orally with aqueous extract of *Garcinia indica* fruit rind at a dose of 200 mg/kg, for acute and subacute studies consequetively. (cyclophosphamide and Vit. E) used in the present study and maximum human dose per day was converted into mice dose. The calculated doses of all the drugs have been shown in **Table no. 2**

Cyclophosphamide powder was dissolved in 20 ml water for injection to prepare 10mg/ml final concentration to accommodate the required dosing in permissible volume. cyclophosphamide was given intraperitonaelly. Standard antioxidant Vit. E was prepared as a suspension by titurating with water and 1% Tweeen 80 and was administered intraperitonially. The test drug (GI EXTRACT) was prepared as a suspension by triturating with distilled water. The test substance was administered orally using mice feeding tube.

Albino mice, 7-12weeks old, weighing 20-25gm of either sex were used for experiments. All mice irrespective of their sex were randomly divided into 6 treatment groups (n=5 each).

Table 3: Treatment schedule for acute and subacute study

Groups	Drug treatment (Dose)	No. Of mice required	
		(acute)	(subacute)
1	Control (normal saline) (8ml/kg)	5	5
2	Cyclophosphamide (40mg/kg)	5	5
3	Cyclophosphamide + Std. Antioxidant (40mg/kg) + (50 IU/kg)	5	5
4	GI extract (200mg/kg)	5	5
5	GI extract + Cyclophosphamide (200mg/kg) + (40mg/kg)	5	5
6	Vit. E (Standard antioxidant) (50 IU/kg)	5	5

A single dose for acute study and repeated doses (once a day for 7 days) for sub acute studies were administered. In both studies 24 hours after the last dose of drug administration, about 0.5ml of blood was collected by retro orbital method to isolate the lymphocytes, which were subjected for comet assay. Later the animals were sacrificed with overdose of ether anesthesia to aspirate the femoral bone marrow for micronucleus assay. The Treatment schedule for acute and subacute study shown in Table 3.

IN VIVO MICRONUCLEUS ASSAY[26]: The in vivo micronucleus test is a method devised primarily for screening chemicals for chromosome breaking effects. The testing procedure, developed and the test drugs in their selected doses were administered intraperitoneally (cyclophosphamide).

a) Extraction of bone marrow from mouse: After sacrificing the animals with excess anesthesia (ether), both femora were removed *in toto*, by cutting through pelvis and tibia. The bones were then freed from muscles fibres by the use of gauze and fingers. By gentle traction, the distal epiphysis was torn off together with the rest of the tibia and the surrounding muscle. The proximal end of femur was carefully shortened with scissors until a small opening to the marrow canal becomes visible. The bone marrow was aspirated with the needle of appropriate size mounted, about 0.2ml normal saline pulled from the tube into a disposable plastic syringe. Then the femur was submerged completely in the normal saline (5ml) contained in a centrifuge tube and squeezed against the tube to prevent the bone from slipping off the needle and subsequently the marrow was aspirated. After several gentle aspirations and flushings, the process was repeated from the distal end of the femur, so the that bone marrow cells get into the serum as a fine suspension and not in the form of gross particles.

b) Preparation of smears: The bone marrow cells were was centrifuged at 1000 revolutions per minute for 5 minutes. The supernatant was removed with Pasteur pipette. If the sediment was large, half a drop of serum was left; if it was minute, all the supernatant was drawn off. The cells in the sediment were carefully mixed by aspiration into the capillary part of a fresh, siliconized Pasteur pipette. A small drop of viscous suspension was put on the end of a slide and spread by pulling the material behind a polished glass slide held at an angle of 45degrees. The size of the droplet chosen was so that all the material is used up at a distance of 2-3 cm. Two slides were prepared per animal and air dried before they were stained.

c) Preparation of May-Gruenwald stain (undiluted): 0.3 gm of May-Gruenwald (MG) powder was weighed & transferred to a conical flask of 200-250 ml capacity. 100ml of methanol was added & the mixture was warmed to 50°C. The flask was then allowed to cool to room temperature and was shaken several times

during day. After standing for 24hr the solution was filtered. It was ready for use.

d) Preparation of Giemsa stain (undiluted): 1 gm of Giemsa powder was dissolved in 54ml of glycerol, 54 ml of methanol was added & filtered. After which, it was ready for use.

e) Staining: Air dried slides were immersed in coplin jar containing undiluted MG stain for 3 min. Then, these slides were transferred to another coplin jar containing diluted MG stain (1:1) and were kept for 2 min followed by then these slides were immersed in coplin jar containing Giemsa diluted (1:6) with distilled water. Then the slides were rinsed in distilled water and blot dried with filter paper. After cleaning the back of slide with methanol and dipped in xylene for 5min. For making the smears permanent cover slip smeared with DPX was mounted on the smear.

f) Analyzing slides: All slides were coded for evaluation. First the slides were screened at medium magnification for regions of suitable technical quality where the cells were well spread, undamaged and perfectly stained. Such regions are normally located in a zone close to the end of the smear. A perfect morphology of the nucleated cells serves as criterion for good quality, even though the nucleated cells are not evaluated in the test. The erythrocytes must be well spread, neither globular nor having slurred contours. Their staining has to be vigorous, red in mature erythrocytes and with a strong bluish tint in the immature forms (PCEs).

At medium to high magnification 2000 polychromatic erythrocytes per animal were screened for the presence of micronuclei. For bone marrow toxicity at least 200 erythrocytes were evaluated per animal for the proportion of PCE in bone marrow. As an important control it is necessary to register, separately, the number of micronucleated mature erythrocytes. Normally, such micronucleated NCE number doesn't exceed 5 per 1000 PCEs. If their incidence exceeds a limit of five in the fields containing one thousand polychromatic erythrocytes the suspicion arises that the preparation contains artifacts resembling micronuclei.

Statistical Analysis: Results were expressed as mean ± S.E.M. The statistical significance of any difference in each parameter among the groups was evaluated by one-way ANOVA, using Tukey's multiple comparisons test as *post hoc* test.

Single Cell Gel Electrophoresis (COMET ASSAY)[27-29]: The SCGE or comet assay combines the simplicity of biochemical techniques for detecting DNA single strand breaks with single cell approach. The power of the assay lies in its ability to evaluate DNA damage and repair in proliferating or non-proliferating cells and to provide insight into intercellular differences in response.

a) Preparation of solutions

Phosphate buffer saline (ca^{++}, Mg^{++} free) for 1000 ml (PBS): 8 gm of sodium chloride, 0.2 gm

of potassium chloride, 11.5gm of disodium orthophosphate and 0.2gm of potassium dihydrogen phosphate was weighed and dissolved in 500 ml of distilled water and then total volume was made up to 1000ml. The pH was adjusted to 7.4, filtered and stored at 4°C.

Low melting agarose (LMA): To prepare 0.5% low melting agaorse, 125mg of agarose was mixed with 25ml PBS and heated until near boiling to dissolve the agarose. The melted gel was stored in 5ml aliquots at 4°C

Normal melting agarose (NMA): To prepare 0.67% normal melting agarose, 167mg agarose was mixed with 25 ml PBS. The gel was heated until boiling to dissolve the agarose. The melted gel was stored in 5ml aliquots at 4°C

Fixing solution(A): To prepare 500ml; 75gm of trichloroacetic acid, 25gm of zinc sulphate and 25gm of glycerol was mixed in 500ml of double distilled water. An ingredient of various Buffer solutions shows in Table 4.

Table 4: Ingredients of various Buffer solutions

Ingredient	Lysis buffer solution	Electrophoresis buffer solution	Neutralizing solution
NaCl	2.5 M	—	—
NaOH	_	300mM	—
EDTA	100mM	1mM	—
Tris HCl	10mM	—	0.4M
DMSO	10%	—	—
Triton X 100	1%	—	—
pH	10	>13	7.5
Temp.	4°C	4°C	4°C

b) Silver staining method

Step I: For the silver staining procedure, best results are obtained using agarose precoated, plain glass slides. Onto a clean, dry, plain about 150µl of hot , 1%NMPA is dropped and smeared in one direction with the help of another plain slide inclined at about 45°C this ensures a uniform thin layer of agarose. Plain slides may also be prepared by dipping the slide into hot 1%NMPA and wiping one side dry.

Caution: (The use of plain slides may be associated with the hazard of gel slippage, which may be prevented by preparing the slides in an air conditioned room adjusted at 25°C

Step II: These slides are allowed to dry at 37°C overnight and may be stored for several weeks in a dust free environment.

Step III: The precoated slides are layered with two layers of LMPA gels, one containing the cells and the other without them, as described earlier, and electrophoresed.

Step IV: After electrophoresis the gel slides were neutralized with 0.4M tris and dried for about an hour at room temperature.

Step V: These slides were then placed in fixing solution (A) for 10 and again washed several times with water.

StepVI: The slides were then allowed to dry at 37°C for at least 1h to a maximum of overnight.

Step VII: The staining solution should cover the slides uniformly. The tray containing the slides is to be shaken very gently throughout the staining procedure of 10-20min to ensure uniform staining. This step may be repeated a couple of times with fresh staining solution until a grayish

colour develop on the slides.

Step VIII: The slides were then transferred to a jar containing the stopping solution (E) for about 5min or until a yellowish brown colour develops. The slides may be stored in a dust free environment for long periods.

Step IX: The slides were then observed under transmission microscope.

c) Analysis of slides: The slides were evaluated by micro inspection system fitted with a CCD camera. Using a stage micrometer and ocular micrometer, the microscope was calibrated to measure comet tail lengths. Comets were first observed in 10x followed by higher magnification. Minimum of 100 comets were scored per animal, with 50 comets scored per replicate slide. Undamaged cells were observed as intact nucleus without a tail and damaged cells had the appearance of a comet. The length of the comet, which is a marker of DNA damage, was measured using an ocular & stage micrometer. Degree of DNA damage for each cell was quantified and is directly proportional to tail length. Since, tail length cannot be directly measured it was calculated by the following formula:

Comet tail length (µm) = maximum total comet length – head diameter.

d) Storage of slides: Slides were stored after dehydrating them by keeping in cold 100% ethanol or cold 100%methanol for 20 min. Air dried the slides were stored in dry area.

Statistical Analysis: All the results were expressed in mean ± S.E.M. In *in vivo* micronucleus assay, DNA damage was measured as percentage of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes (%MnPCE). Bone marrow cytotoxicity was assessed in terms of %PCE in 200 erythrocyte count. In comet assay, cell viability of lymphocytes was reported as percentage of viable cells, and comet tail length, which is proportional to DNA damage. The statistical significance of any difference in each parameter among the groups was evaluated by one-way ANOVA, using Tukey's multiple comparisons test as *post hoc* test.

Table 5 : Results of qualitative chemical investigation of *Hibiscus Rosa-sinensis*

Sr.No.	Chemical Test	*EthanolicExtract(GI)*
1	**Tests for Carbohydrates** Molish's test	+
A)	**Tests for reducing sugars** a)Fehling's test b)Benedicts test	+ +
B)	**Test for Monosaccharides** a) Barfoeds test	-
C)	**Tests for Hexose sugars** a)Cobalt chloride test	+
D)	**Tests for Non-reducing polysaccharides (starch)** a)Iodine test b)Tannic acid test for starch	- -

2	**Tests for Proteins**: a)Biuret test b)Millions test c)Lead acetate test	+ - +
3	**Tests for Steroids:** a)Salkowski reaction b)Liebermann-Burchard reaction c)Liebermann reaction	+ + +
4	**Tests for Flavanoids:** a)Ferric Chloride test b)Shinoda test c)Alkaline reagent test	+ + +

I) Invitro Antioxidant Properties Of *Hibiscus Rosa-Sinensis*[30-33]

Radical Generation and Scavenging Studies

1) Scavenging of stable DPPH and ABTS free radicals by HR (***Hibiscus Rosa-sinensis***) (Graph No. 1): The DPPH radical is widely used as a model system to investigate the free-radical scavenging activities of several plant extracts. Figure 1. shows the dose-response curve for Hibiscus Rosa-sinensis on DPPH radical scavenging activity. Hibiscus Rosa-sinensis scavenged the DPPH radicals in a concentration-dependent manner with the maximum scavenging activity of 77.80% at 200 µg/ml attaining saturation with further increase in the Hibiscus Rosa-sinensis concentration. Similarly Hibiscus Rosa-sinensis demonstrated ABTS radical scavenging activity in a dose-dependent manner with 52.7% at 200 µg/ml HRS concentration attaining saturation with higher concentrations **Graph no.1.**

2)Nitric oxide, radical scavenging activity of Hibiscus Rosa-sinensis.: **Graph no.1** also show the potential of Hibiscus Rosa-sinensis in scavenging nitric oxide radical in a concentration-dependent manner (50–350 µg/ml) with the maximum scavenging activity of 52.9%, at 200 µg/ml attaining saturation with further increase in the Hibiscus Rosa-sinensis concentration.

3)The effect of Hibiscus Rosa-sinensis on lipid peroxidation: The addition of Hibiscus Rosa-sinensis to the reaction mixture resulted in a moderate dose-dependent inhibition of lipid peroxidation with the maximum inhibition of 56.7% at 200 µg/ml and saturated thereafter **Graph no 2.**

II) Protection Of Cyclophosphamide Induced Genotoxicity By Hibiscus Rosa-Sinensis

There were no mortality observed for the control or drug treated groups. For micronucleus assay mean values for the proportion of polychromatic erythrocytes per total erythrocytes was calculated to see any toxicity. The incidences of micronucleated polychromatic erythrocytes (MnPCE) per 2000 polychromatic erythrocytes (PCE) were scored in all the drug treated groups both in acute as well as sub acute studies (**Table no 6).** As expected, the Cyclophosphamide group induced statistically significant increase in % MnPCE as compared

with that of the normal saline (negative control) with a mean and standard error of **4.310 ± 0.134** and **8.310 ± 0.232** % respectively at both 24 hr and 7days harvest time. Whereas CYP+HR group showed protection against cyclophosphamide induced genotoxicity by a significant decrease in %MnPCE with a mean and standard error of **1.470 ± 0.117** and **4.38 ± 0.154** % as compared with that of CYP group(positive control). Whereas it did not show statistically significant decrease in %Mn PCE as compared with that of the standard group (CYP+VIT E) GI treated group by itself did not showed any genotoxic effect ie. no significant increase in% MnPCE as compared to that of CYP group. Accessing the genotoxicity of the standard ie. VIT E no significant results were found regarding its genotoxicity. Silver stained slides of comet assay (SCGE) were studied for the presence of comets. The parameter studied was comet tail length (µm) assessed with the help of ocular and stage micrometer. Incidence of viable lymphocytes (unstained with trypan blue) was more than 95% in all the groups. Cyclophosphamide group showed significant (P<0.01) increase in tail length with 24.90 ± 0.674 & 26.00 ± 0.649 in acute and sub-acute studies respectively when compared to the saline group (**Table no 7),** whereas CYP+ HR group showed significant decrease in comet tail length with a mean and standard error of **10.150 ± 0.415** and **11.30 ± 0.260** % as compared with that of CYP group (positive control), and thus rendered protection

Table 6 : Incidence of % MnPCE (in 2000PCE) in acute & sub-acute studies.

Serial number	Treatment groups mg/kg (n =5)	Acute study		Sub-Acute study	
		% MnPCE Mean ± S.E.M	%PCE Mean ± S.E.M	%MnPCE Mean ± S.E.M	%PCE Mean ± S.E.M
1.	NS (Negative Control 8ml/kg	0.460±0.022	58.60 ±1.364	0.4500±0.050	55.50 ± 1.000
2.	CYP (Positive Control) 40mg/kg	4.310±0.134*	56.20 ± 1.158	8..130±0.232*	53.40 ± 1.269
3.	CYP+GI 40+200mg/kg	1.470±0.117**	55.90 ± 2.033	4.38± 0.154**	54.70 ± 1.347
4.	GI 200mg/kg	0.380±0.029	55.50 ± 1.313	0.4500±0.054	55.50 ± 1.492
5.	CYP+ Vit. E (standard) 40+ 50IU mg/kg	1.540±0.093***	56.50 ± 1.225	4.450±0.0763***	54.70 ± 1.663
6	Vit. E 50 IU	0.3100± O.027	55.70±1.224	0.4400±O.O45	55.52±1.196

One-way ANOVA, F = 326.1 (acute study); 655.5 (sub-acute study)
*P<0.001 when compared with negative control.
** P<0.001 when compared with positive control.
*** P<0.001 when compared with positive control

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Table 7 : Comet tail length (µm) in acute & sub acute study

S. no.	Treatment group mg/kg (n =5)	Acute study Mean ± S.E.M	Sub-Acute study Mean ± S.E.M
1.	NS (Negative Control) 8ml/kg	3.920 ± 0.2318	4.930 ± 0.2295
2.	CYP (Positive Control) 40mg/kg	24.90 ± 0.674*	26.70 ± 0.649
3.	CYP + HR (40mg/kg+200mg/kg)	10.150 ± 0.415**	11.30 ± 0.260
4.	HR 200mg/kg	3.450 ± 0.311	4.300 ± 0.290
5.	CYP + Vit. E (40mg/kg+ 50IU)	10.200± 0.489***	11.400 ± 0.636
6	Vit. E 50 IU	3.520± 0.2707	3.90± 0.2211

One-way ANOVA, F = 377.5 (acute study); 393.7 (sub-acute study)
* P<0.001 when compared with negative control.
** P<0.001 when compared with positive control.
***P<0.001 when compared with positive control

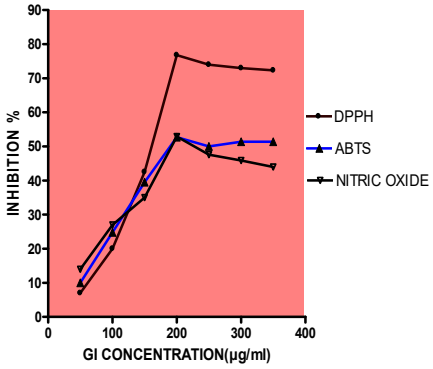
against cyclophosphamide induced genotoxicity. Whereas (CYP+ HR) group did not show statistically significant decrease in tail length as compared with that of the standard group (CYP+VIT E) , GI treated group by itself did not showed any genotoxic effect ie. no significant increase in comet tail length as compared to that of CYP group. Acessing the genotoxicity of the standard ie. VIT E no significant results were found regarding its genotoxicity.

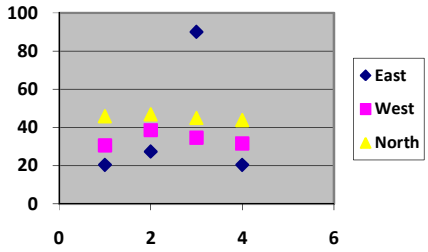
Comparing acute and subacute study groups, it was found that the extract rendered protection against cyclophosphamide induced genotoxicity, Significant (P<0.001) decrease in comet tail length and % MnPCE was observed in both acute & subacute studies of CYP+ HR group, whereas there was no significant comparision with that of the standard (VIT E). HR treated groups did not show any significant genotoxic effect, and is not genotoxic by itself. The standard antioxidant was also found to be non genotoxic.

GRAPHICAL REPRESENTATIONS

I) In-vitro antioxidant studies.

a) Free radical scavenging activity.





Graph No. 1. Interaction of various concentrations of HR aqueous extract with DPPH **(2, 2-diphenyl-2-picryl hydrazyl)** (filled circle), ABTS **(2,2-azinobis(3-ethylbenzothiazoline–6–sulfonicacid)** [filled triangle], and nitric oxide [open triangle]

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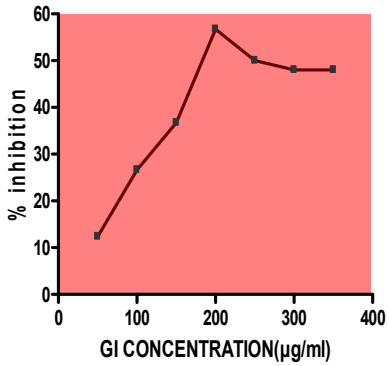
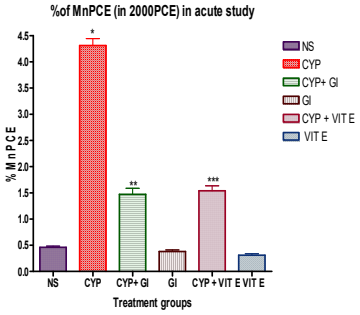
B) Inhibition of Lipid Peroxidation



Graph No. 2. Inhibition of lipid peroxidation by various concentrations of aqueous extract of HR

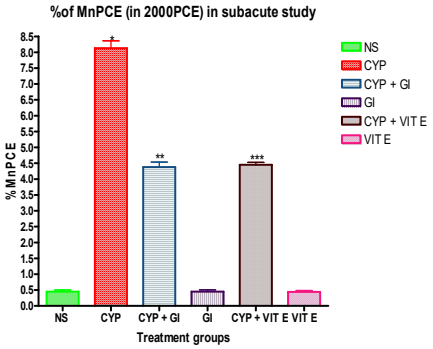
b)Genotoxicity studies.

a) In Vivo Micronucleus Assay

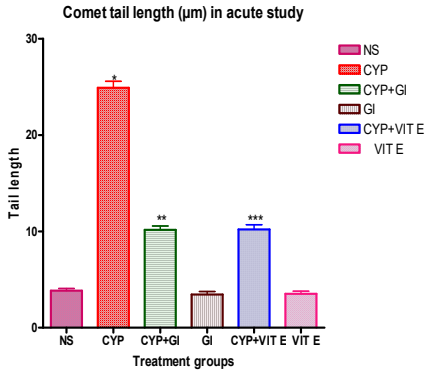


Graph no.3. Effect of aqueous Hibiscus Rosa-sinensis fruit rind extract on modifying cyclophosphamide induced genotoxicity. Each bar represents the %MnPCE (acute study) mean ± SEM from 5 animals in each group. *P<0.001 when compared with negative control. ** P<0.001 when compared with positive control. *** P<0.001 when compared with positive control.

Graph No. 4. Effect of aqueous Hibiscus Rosa-sinensis fruit rind extract on modifying cyclophosphamide induced genotoxicity. Each bar represents the %MnPCE(sub acute study)



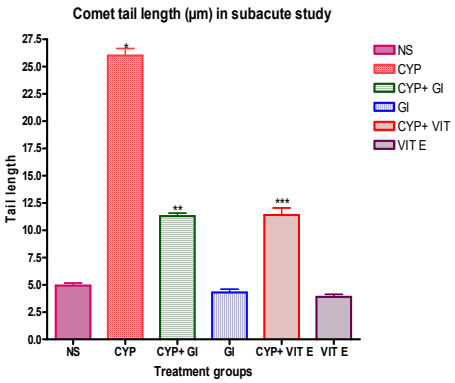
mean ± SEM from 5 animals in each group. *P<0.001 when compared with negative control. ** P<0.001 when compared with positive control. *** P<0.001 when compared with positive control.



Graph No. 5. Effect of aqueous Hibiscus Rosa-sinensis fruit rind extract on modifying cyclophosphamide induced genotoxicity. Each bar represents the comet tail length (acute study) mean ± SEM from 5 animals in each group. *P<0.001 when compared with negative control. ** P<0.001 when compared with positive control. *** P<0.001 when compared with positive control.

Graph No. 6. Effect of aqueous Hibiscus Rosa-sinensis fruit rind extract on modifying cyclophosphamide induced genotoxicity. Each

bar represents the comet tail length (sub acute study) mean ± SEM from 5 animals in each group.*P<0.001 when compared with negative



control. ** P<0.001 when compared with positive control. *** P<0.001 when compared with positive control.

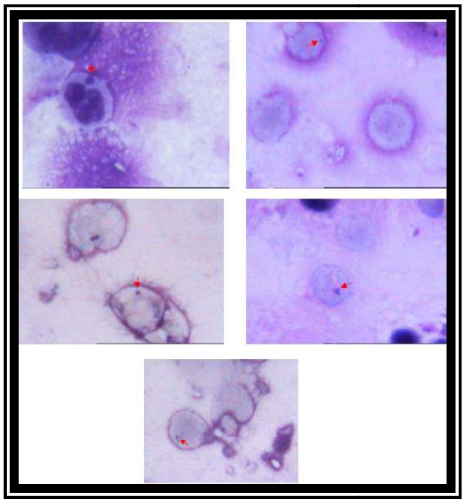
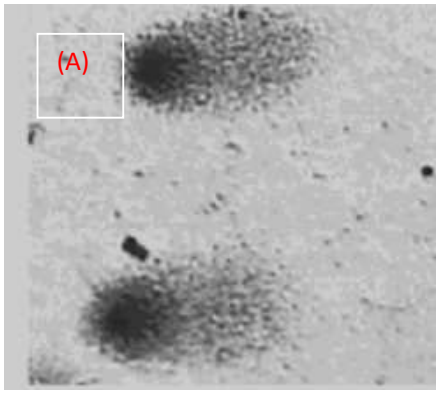


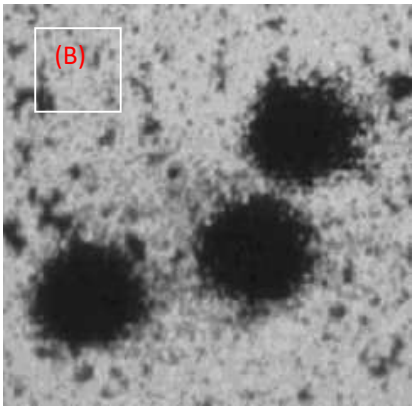
Fig. 7. Photomicrographs of Micronuclei (red arrows) in mice bone marrow

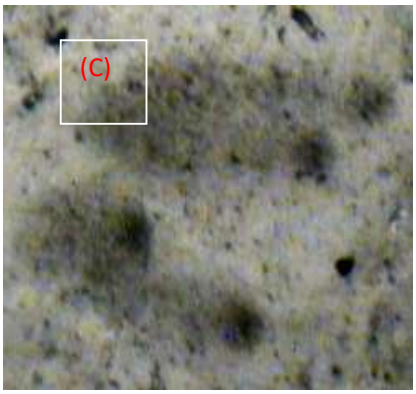
Various level of DNA damage seen in various groups.(comet assay)

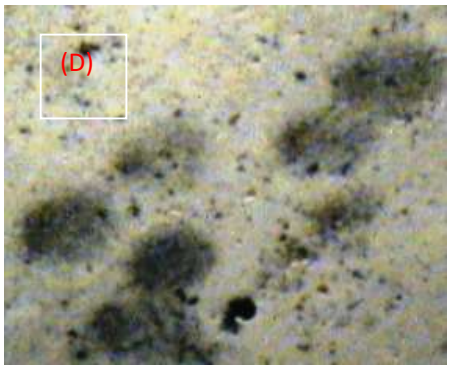
(A) Negative control group (normal saline) no damage is seen

(B) & (C) Positive control group (Cyclophosphamide) maximal damage is seen









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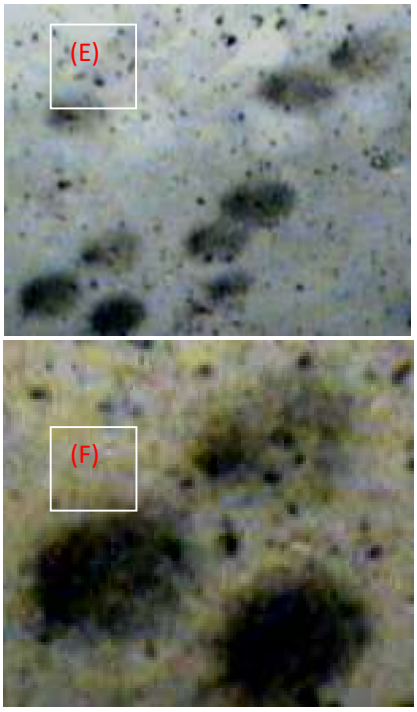
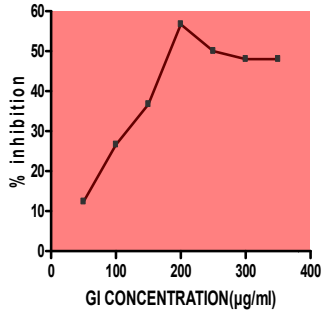


Fig. 8: Various level of DNA damage seen in various groups.(comet assay)

(D) Cyclophosphamide + VIT E (intermediate damage is seen)
(E) Cyclophosphamide + HR extract (intermediate damage is seen)
(F) HR alone (no damage is seen)



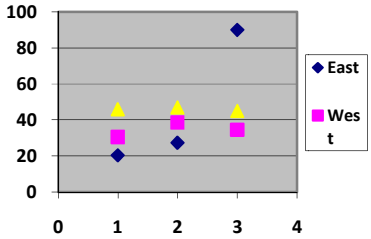


Table 8: Results of qualitative chemical investigation *Garcinia Indica*

Sr.No.	Chemical Test	*EthanolicExtract(GI)*
1	**Tests for Proteins**: a)Biuret test b)Millions test c)$(CH_3COO)_2$ test	 + - +
2	**Tests for Carbohydrates**	+
A)	**Tests for reducing sugars** a)Fehling's test b)Benedicts test	+ +
B)	**Tests for Hexose sugars** a)$COCl_2$ test	+
C)	**Tests for Non-reducing polysaccharides (starch)** a)I_2 test b)Tannic acid test	 - -
D)	**Test for Monosaccharides**	-
3	**Tests for Flavanoids:** a)$FeCl_3$ test b)Shinoda test c)Basic (Alkanity) reagent test	 + + +
4	**Tests for Steroids:** a)Salkowaski test b) Burchard-Liebermann reaction c)Liebermann nitroso reaction	 + + +

I) Invitro Antioxidant Properties of *Garcinia Indica* [34]

Radical Generation and Scavenging Studies

1) Scavenging of stable DPPH and ABTS free radicals by GI: The DPPH radical is widely used as a model system to investigate the free-

radical scavenging activities of several plant extracts. It shows the dose-response curve for GI on DPPH radical scavenging activity. GI scavenged the DPPH radicals in a concentration-dependent manner with the maximum scavenging activity of 73.80% at 200 µg/ml attaining saturation with further increase in the GI concentration. Similarly GI demonstrated ABTS radical scavenging activity in a dose- dependent manner with 55.7% at 200 µg/ml HRS concentration attaining saturation with higher concentrations.

2) Nitric oxide, radical scavenging activity of GI. **It** show the potential of GI in scavenging nitric oxide radical in a concentration-dependent manner (70–300 µg/ml) with the maximum scavenging activity of 55.9%, at 200 µg/ml attaining saturation with further increase in the GI concentration.

3) The effect of GI on lipid peroxidation: The addition of GI to the reaction mixture resulted in a moderate dose-dependent inhibition of lipid peroxidation with the maximum inhibition of 57.7% at 200 µg/ml.

II) Protection of Cyclophosphamide induced Genotoxicity by GI: There were no mortality observed for the control or drug treated groups. For micronucleus assay mean values for the proportion of polychromatic erythrocytes per total erythrocytes was calculated to see any toxicity. The incidences of micronucleated polychromatic erythrocytes (MnPCE) per 2000 polychromatic erythrocytes (PCE) were scored in all the drug treated groups both in acute as well as sub acute studies (**Table no 9).** As expected, the Cyclophosphamide group induced statistically significant increase in % MnPCE as compared with that of the normal saline (negative control) with a mean and standard error of **4.210 ± 0.144** and **8.510 ± 0.242** % respectively at both 24 hr and 7days harvest time. Whereas CYP+GI group showed protection against cyclophosphamide induced genotoxicity by a significant decrease in %MnPCE with a mean and standard error of **1.460 ± 0.127** and **4.36 ± 0.144** % as compared with that of CYP group(positive control). Whereas it did not show statistically significant decrease in %MnPCE as compared with that of the standard group (CYP+VIT E) GI treated group by itself did not showed any genotoxic effect ie. no significant increase in% MnPCE as compared to that of CYP group. Accessing the genotoxicity of the standard ie. VIT E no significant results were found regarding its genotoxicity.

Silver stained slides of comet assay (SCGE) were studied for the presence of comets. The parameter studied was comet tail length (µm) assessed with the help of ocular and stage micrometer. Incidence of viable lymphocytes (unstained with trypan blue) was more than 95% in all the groups. Cyclophosphamide group showed significant ($P<0.01$) increase in tail length with 24.70 ± 0.684 & 25.00 ± 0.659 in acute and sub-acute studies respectively when

compared to the saline group (**Table no 9),** whereas Whereas CYP+GI group showed significant decrease in comet tail length with a mean and standard error of **10.250 ± 0.425** and **12.30 ± 0.250** % as compared with that of CYP group(positive control), and thus rendered protection against cyclophosphamide induced genotoxicity. Whereas (CYP+GI) group did not show statistically significant decrease in tail length as compared with that of the standard group (CYP+VIT E) , GI treated group by itself did not showed any genotoxic effect ie. no significant increase in comet tail length as compared to that of CYP group. Acessing the genotoxicity of the standard ie. VIT E no significant results were found regarding its genotoxicity.

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Table 9 : Incidence of % MnPCE (in 2000 PCE) in acute & sub-acute studies.

S.no	Treatment groups mg/kg (n =5)	Acute study		Sub-Acute study	
		% MnPCE Mean ± S.E.M	%PCE Mean ± S.E.M	%MnPCE Mean ± S.E.M	%PCE Mean ± S.E.M
1.	NS (Negative Control 8ml/kg	0.440±0.022	58.50 ±1.364	0.4500±0.050	55.50 ± 1.000
2.	CYP (Positive Control) 40mg/kg	4.410±0.134*	46.20 ± 1.158	8.140±0.242*	53.40 ± 1.269
3.	CYP+GI 40+200mg/kg	1.470±0.117**	54.90 ± 2.043	4.38± 0.154**	54.70 ± 1.347
4.	GI 200mg/kg	0.380±0.029	55.50 ± 1.313	0.4500±0.057	55.50 ± 1.472
5.	CYP+ Vit. E (standard) 40+ 50IU mg/kg	1.540±0.093***	54.50 ± 1.224	4.450±0.0743***	54.40 ± 1.653
6	Vit. E 50 IU	0.5100± O.025	55.70±1.234	0.4500±O.O45	54.52±1.185

One-way ANOVA, F = 325.1 (acute study); 645.5 (sub-acute study)
*P<0.001 when compared with negative control.
** P<0.001 when compared with positive control.
*** P<0.001 when compared with positive control

Table 10: Comet tail length (µm) in acute & sub acute study

S.no	Treatment group mg/kg (n =5)	Acute study Mean ± S.E.M	Sub-Acute study Mean ± S.E.M
1.	NS (Negative Control) 8ml/kg	3.820 ± 0.2318	4.920 ± 0.2295
2.	CYP (Positive Control) 40mg/kg	24.80 ± 0.684*	26.70 ± 0.549
3.	CYP +GI 40mg/kg+200mg/kg	10.150 ± 0.418**	12.30 ± 0.260
4.	GI 200mg/kg	3.480 ± 0.311	4.500 ± 0.290
5.	CYP + Vit. E 40mg/kg+ 50IU	10.204± 0.488***	12.400 ± 0.526
6	Vit. E 50 IU	3.420± 0.2807	2.90± 0.2212

Comparing acute and subacute study groups, it was found that the extract rendered protection against cyclophosphamide induced genotoxicity, Significant (P<0.001) decrease in comet tail length and % MnPCE was observed in both acute & subacute studies of CYP+GI group, whereas there was no significant comparision with that of the standard (VIT E). GI treated groups did not show any significant genotoxic effect, and is not genotoxic by itself. The standard antioxidant was also found to be non genotoxic.

CONCLUSION

Garcinol from Garcinia indica fruit reported the free radical Scavenging activity and antiulcer activity. Orally administered garcinol prevented acute ulceration in rats induced by indomethacin and water immersion stress caused by radical formation. These results suggested garcinol might have potential as a free radical scavenger and clinical application as an antiulcer drug.

The methanolic flower extract of hibiscus rosa sinensis was evaluated with respect to its genotoxic potential through micronucleus assay in Balb/c mice. The frequency of micronuclei in groups of animals treated with hibiscus rosa sinensis showed no differences as compared to the negative control(vehicle); therefore, it is considered that the hibiscus rosa sinensis showed no genotoxic activity in the micronucleus test.

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Conflict of Interest

The authors declare that they have no conflict of interest