

Acute Toxicity and Genotoxic Activity of *Hibiscus rosa sinensis* Flower Extract

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ABSTRACT

The use of herbal extracts requires toxicological and genotoxic evaluations to establish and verify safety before being added to human cosmetic, pharmaceutical medicine, or alimentary products. *Hibiscus rosa sinensis* flowers have been used in traditional medicine as treatment for several diseases. In this work, 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.

Keywords: Genotoxicity, *Hibiscus rosa sinensis*, Acute toxicity, Micronucleus assay.

INTRODUCTION

Hibiscus rosa sinensis L (Malvaceae) is an ornamental plant, native to China grown as an evergreen herbaceous plant. For the medicinal purpose, red flower variety of hibiscus is mostly preferred¹. It possesses various pharmacological activities such as radical scavenging, antipyretic and anti-inflammatory^{13,8}. Leaves and flowers of Hibiscus show potential against hair fall, and hypoglycemic activity^{14,15} and also having healing activity against ulcers⁴. The flowers also possess anti-implantation and antispermatogenic activities^{5,9}. It is also being known to contain an anthocyanin

pigment, cyanidin diglucosides, carotene, thiamine, riboflavin, niacin and ascorbic acid. That is why it is traditionally used as antifertility agent from ancient time of². The extracts of *Hibiscus rosa sinensis* have also been shown a protective effect against the tumour promotion stage of cancer development¹⁶ In this study, we evaluate the genotoxic effect of *Hibiscus rosa sinensis* extract *in vivo*, by induction of micronuclei in blood polychromatic erythrocytes of Balb/c mice.

MATERIAL AND METHOD

Plant Material

The flowers of *Hibiscus rosa sinensis* were purchased from local commercial source of Lucknow. The plant material was authenticated in the botany Division at CSIR- CDRI, Lucknow.

Hibiscus rosa sinensis flower Extract

Methanolic extract of *Hibiscus rosa sinensis* flower was obtained through soxhlet reflux equipment and evaporated on a rotary evaporator¹¹. Dried flowers (2000 gm) were powdered in a laboratory mill, and macerated with freshly distilled methanol till exhaustion. After filtration, extract was concentrated under vacuum at 35°C.

Animals

Fifty-five, eight-week-old male Balb/c mice (25 ± 2 g) were obtained from National Laboratory Animal Center (NLAC), Central Drug Research Institute, Lucknow (India) prior to experiment animals were allowed to acclimatize at controlled environment ($25 \pm 2^\circ\text{C}$) for 7 days. They were kept at 30–60% relative humidity with 12 h light and dark cycle. The animals were fed a standard rodent pellet diet and water *ad libitum*. Animal studies were conducted according to the regulations of the Institute Animal Ethics Committee, IAEC No. - IAEC/2012/86.

Acute Toxicity Test

For the determination of median lethal dose (LD_{50}) of *Hibiscus rosa sinensis* flower extract, five groups of animal (each group contained 5 mice) were administered oral gavage one by one at different doses consisting of 100, 200, 400, 800, and 1600 mg/kg. Mortality was recorded 24 hours after the administration of the extract. Animals were observed during one week to

detect signs of delayed toxicity.

Genotoxicity Test

Genotoxicity was induced by the administration of cyclophosphamide in six group of animal (each group contained 5 mice individually). To investigate the protective effect of *Hibiscus rosa sinensis* (HRS) against the Genotoxicity induced by CP, group 1 mice received drinking water (1 ml per day by gavage) for 15 consecutive days and were treated intraperitoneally (i.p.) on day 15 with 0.9% NaCl. Group 2 received drinking water (1 ml per day by gavage) for 15 days, and mice were treated with CP (20mg/kg_1 body weight) on day 15. Groups 5 and 6 received extract of *Hibiscus rosa sinensis* (group 4, 200mg/kg body weight; group 5, 400mg/kg body weight) by gavage for 15 days before treatment with CP on day 15. Groups 3 and 4 received only treatments with *Hibiscus rosa sinensis* extract during 15 consecutive days and were i.p. -treated on day 15 with 0.9% NaCl, to investigate a possible effect on spontaneous genotoxic damage. After 24 hours administration of last dose, animals were sacrificed by cervical dislocation without anesthesia to avoid possible alterations in the DNA damage analysis. Femur bones were removed clean by cotton and the bone marrow cells were flushed from both femurs in FCS (fetal calf serum). After centrifugation (1000 rpm for 5 min), the supernatant is then removed with the help of a Pasteur pipette. The residue left behind is mixed thoroughly with the help of Pasteur pipette and smeared onto a clean slide, coded for blind analysis, air-dried. These air-dried smear slides were stained with May-Grunwald and Giemsa stains to detect micronucleated polychromatic erythrocytes (mn-PCE) and normochromatic erythrocytes. For each animal, two slides were prepared and 2000 polychromatic erythrocytes (PCE) were counted to

determine the frequency of mn-PCE using light microscope (Olympus BH-2 10 x 100, Tokyo, Japan). Genotoxicity and antigenotoxicity were assessed by the frequency of mn-PCE and the percentage reduction in the frequency of mn-PCEs as compare to control.

Statistical Analysis

All results are expressed as mean \pm S.D. Data were analyzed with one-way ANOVA. Statistically significant effects were further analyzed. The statistical significance was determined at $p < 0.05$.

RESULTS

Acute Toxicity

Administration of *Hibiscus rosa sinensis* flower extract at doses of 100, 200, 400, and 800, mg/Kg did not produce any significant changes in behavior, skin effect, breathing, defecation, postural abnormalities, impairment in food intake and water consumption and yellowing or loss of hair. But at dose 1600 mg/kg showed 20% mortality.

Genotoxicity Test

The potential genotoxic effect of *Hibiscus rosa sinensis* extract *In vivo* and its ability to protect against CP-induced DNA damage are shown in Fig. 1. CP alone induced a clear increase in mn frequency ($P < 0.001$). The results also showed that the frequencies of mn in PCE of animals treated only with the extract are not different from those of untreated controls. When we analyzed the potential protective effect of *Hibiscus rosa sinensis* extract we observed that pre-treatment with *Hibiscus rosa sinensis* extract led to the statistically significant reduction ($P < 0.001$) in the frequency of mn in PCEs. The reduction was between 61.64 and 67.80%, related to increasing dose of *Hibiscus rosa sinensis*

extract (200mg/kg body weight and 400mg/kg body weight). These results suggest that *Hibiscus rosa sinensis* extract provides protection against the genotoxicity of CP.

DISCUSSION

Hibiscus rosa sinensis shows remarkable medicinal properties. Several previous studies showed that *Hibiscus rosa sinensis* is a good anti-fertility^{22,17,5,6}. Antiovolatory⁹ anti-spermatogenic, androgenic^{10,12}, analgesic, anti-inflammatory¹³, wound healing¹⁹ and antidiabetic²⁰ agent. Some *in-vitro* studies also described that *hibiscus rosa sinensis* show good *in-vitro* antioxidant activity³. So that the present study have been designed for the assessment of antigenotoxic potential of this plant.

Oxidative DNA damage can play a significant role in mutagenesis, cancer, aging and other human pathologies, decreasing oxidative stress seems to be the best possible strategy, achieved by eating antioxidants rich food and/or by taking supplements containing polyphenols. The example is plant extracts⁷. The evaluation of micronucleus frequencies *in vivo* is one of the primary genotoxicity tests recommended internationally by regulatory agencies for product safety assessment²¹.

The present study was planned to evaluate the influence of *hibiscus rosa sinensis* (HRS) flower extract on oxidative radicals and CP induced genotoxicity. In this study pretreatment with oral HRS extract (200mg/Kg and 400mg/kg) in CYP induced genotoxic mice showed significant dose dependent reduction in % mn-PCE. Therefore, results of the present study clearly indicate that HRS when co-administrated with cyclophosphamide and on its own conferred protection against genotoxic effects. However, previous study have shown hibiscus rosa sinensis to posses

chemo preventive action in croton oil induced carcinoma model, and most of the anti-cancer drugs including cyclophosphamide are known to induce DNA damage¹⁶ by means of generation of free radicals. *Hibiscus rosa sinensis* itself did not show any genotoxic symptoms at dose 200 and 400mg/kg body wt. According to present study the *hibiscus rosa sinensis* shows good antigenotoxic activity by lowering the no. of mn-PCEs, induced by CP treatment at does 20mg/kg body weight IP.

CONCLUSION

The methanolic extract of the *Hibiscus rosa sinensis* showed acute toxicity at the concentration of 1600 mg/kg. *In vivo* genotoxicity on peripheral blood cells of the flower extract was not observed. However, this study needs to be supported with experimental toxicity studies using isolated compounds. The lack of *In vivo* genotoxic activity of the extract allows us to hope that the *Hibiscus rosa sinensis* flower extract could be used as a possible pharmaceutical material.

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Table 1. Number of cells analyzed, percentage of reduction and micronucleated polychromatic erythrocytes (mn-PCEs) bone marrow from mice treated with *Hibiscus rosa sinensis* and cyclophosphamide

Treatment	Number of cells analyzed	Number of mn-PCEs	Reduction(%)
Control 0.9% NaCl	10000 (n=5)	10	—
CP 20mg/kg	10000 (n=5)	156*	—
200mg/kg HRS	10000 (n=5)	12	—
400mg/kg HRS	10000 (n=5)	14	—
200mg/kg HRS+ CP20mg/Kg	10000 (n=5)	66	61.64
400mg/kg HRS+ CP20mg/Kg	10000 (n=5)	57	67.80

* P<0.001, positive control vs other groups (Student's t-test).

mn-PCEs- micronucleated polychromatic erythrocytes

Data of each group was pooled (n=5).

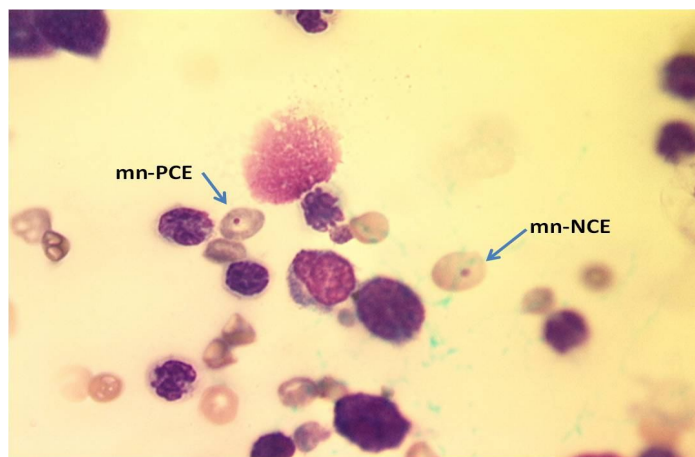


Figure 1. Photomicrograph showing micronucleated polychromatic erythrocyte (mn-PCEs) and micronucleated normochromatic erythrocyte (mn-NCEs)