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Anticlastogenic Activity of Hibiscus and Green Tea Upon Human Lymphocyte Chromosomes

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ABSTRACT

The present work aims at investigating the capability of Hibiscus and green tea extracts in reducing or decreasing the DHA damage induced by the drug indexan as a well known positive control. Human lymphocyte genome was chosen and employed to achieve such a purpose. Two genotoxic bioassays were employed .they are: 1- In vitro induction of SCE Sister chromatid exchange2- Chromosomal aberrations the obtained results, clearly showed that both extracts was proven to be capable in decreasing or reducing micro as well as macro DNA damage. This results, however, presented that the tested extracts are positive as anticlastogonic agents.

INTRODUCTION

Genotoxins are agents specifically producing genetic alterations at sub-toxic exposure levels which result in organisms with altered hereditary characteristics.

Depending upon the developmental stage of an individual, a genotoxin can exert teratogenic effect or cause mutations not only in somatic but also in germinal cells.

Mutational damage results in situation where not only an exposed person has the possibility of deleterious effects but also his progeny generation upon generation. A cytogenetic technique currently in wide use is the analysis for SCEs. This phenomenon was originally observed by Taylor in 1958, but analysis for SCEs on a routine basis only became possible following the development of simple staining techniques that differentiate sister chromatids. SCE analysis appears to be a very good screening tool, for evaluation of primary genetic damage induced by contaminants and / or pollutants.

The ideal genetic assay for occupational monitoring would be rapid, inexpensive, highly objective, and predictive. One technique, SCE detection, appears to have most of these desired properties.

The SCE method using human lymphocytes recovered from test populations is currently undergoing numerous trial studies, and the result appear promising. Early studies on hospital patients receiving chemotherapeutic alkylating agents showed increased SCE. (Seehy,2007) Another pilot study with actual occupational exposures demonstrated a significant elevation of SCEs in a population of petroleum workers. The former results were not surprising, since many chemotherapeutic drugs are suspect human mutagens and carcinogens on the basis of in vitro studies (Matheson et. al., 1978).

Environmental toxicology aims at disclosing the capability of environmental toxicants in causing DNA damage and on the health of all organisms and on the different compartments of the environment. Its concern involves the fact that human survival depends on the preservation of other animal and plant species and on the environmental resources such as clean air, food and water, which are menaced mostly by anthropogenic chemicals that alter living organisms and ecological processes. Therefore, ecotoxicology deals with two orientations: regulation and research (Seehy, 2003).

In recent years, there has been growing concern on the deleterious effects that many chemicals may have on male reproduction. These substances may act as testicular toxicants and correspond to different compounds, which are related to social habits, life conditions, working hazards or use of drugs and medicines (Brusick, 1986).

The increase of environmental chemical pollution becomes evermore apparent when one considers that today there are 4 million organic environmental contaminants, and this number increase in 100 % yearly. Globally, there are 100.000 products annually manufactured in the order of 200 million tons. Per year. They contaminate 30% of the waters and 70% of the general environment. Therefore this work was carried

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out to investigate the possible anti clastogenic effects of Hibiscus and green tea extract upon human lymphocyte genome.

MATERIALS AND METHODS

In vitro induction of sister chromatid exchanges in human lymphocytes:

Heparinized venous blood was collected from normal healthy adults. Human karyotyping medium purchased from GIBCO (USA) was used in this genotoxic bioassay. In order to study the frequency of the sister chromatid exchange in human chromosomes in response to the tested compound-1 100 ug BrdU were added 8 hr before the treatment of culture with the tested compound. The cultures were incubated in tightly sealed tubes at 37 C for 72 hr. Before harvesting at 70 hr 0.1 ml colcemid was added to each culture and incubation was continued for 2 hr. Heparinized venous blood was collected from normal healthy adults.

The standard leucocyte cultures used in this investigation consisted of Minimum Essential Medium (Eagle) with L-Glutamine (SIGMA) supplemented with penicillin-streptomycin (10000 units – 10000mg/ml). For each 10 ml of this medium, 0.5 ml whole, blood, 0.25 ml phytohemaglutinin (SIGMA) were added. The cultures were incubated in tightly sealed tubes at 37 ;C for 72 hours. The proper concentration of the tested compound was added to the four cultures. For each tested concentration Four culture were employed. 24 houres after the incubation positive control and the prope concentration was added.

Two hours prior to harvest 0.1 ml colcemid (SIGMA) was added to 103 Ml EMS in 10 ml stenile water and / 25 mg CP per 1000 ml were used as positive control each tested concentration and then the cultures were reincubated for 2 hours.

The method decribed by Schwazacher (1974) was used in order to prepare the metaphases. As The cultures were centrifuged for 8 min at 1200 rpm, the supernatant was discarded and the cell pellet was resuspended with last drop of supernatant, then about 8 ml of prewormed (37C) hypotonic (0.075 M KCL) were added, allowed to stand for 10 min at 37C, centrifuged for 8 min at 1200 rpm, the cell pellet was fixed for 1 hr in about 8 mL freshly prepared fixative fluid (3 parts methanol : 1 part glacial acetic acid) and centrifuged. The cell pellet was fixed three times : 20 min each.

Staining was carried out using 10% Giemsa (pH 6.8) for 5 min., slides were then air dried and chromosomes were examined for deletion, break, gap.. etc. (Brusick, 1986). For each concentration 100scorable metaphase cells were examined for the different types of chromosomal aberrations.

In order to investigate the effect of the used concentrations upon cell proliferation a metaphase index as well as mitotic index (from untreated cultures with colcemid) based on at least 1000 counted cells were recorded.

Preparation of metaphase chromosomes:

The method described by Schwarzacher (1974) was used as follows: Staining was performed by the method of Goto et. al. (1976). The slides were stained with 50 mg\ml of Hoechest 33258 dye in distilled water, pH 7.0 for 10 min (protected from light). The slides were then rinsed in water , and covered by a layer of Mc llvaines buffer [add 18 ml of solution A (1.92% citric acid) to 82 ml of solution B (2.42% disodium phosphate A) and adjust the pH to 7.0 or 7.5 with further mixing], mounted by cover slip and subjected to light with intensity <= 400nm, at a distance of about 2 inches for 20 min. during this time, slides were placed on a wormer tray at 50 C. The slides then were rinsed in distilled water and immersed in 4 % Giemsa dye, rinsed again in water and allowed to dry for subsequent light microscope analysis.

Screening of slides and analysis:

Scanning slides for metaphase spreads was conveniently accomplished with a 25 X magnification objective, and analysis was with a 100 X objective. For control of bias, all prepared slides were coded prior to scoring. There are two ways for counting sister chromatid exchange frequencies i.e., (1) from the microscope images of second division cells, (2) the cells may be photographed and SCE frequencies are counted from the microscope images. An interstial exchanged segment was counted to be 2 SCEs.

Usually, wide ranges of SCE values were encountered specially in treated cells, and then the analysis of variance using F-test was applied. To evaluate the differences in mean SCE frequencies between treated and control groups, Duncun's multiple range test was used.

RESULTS

Table (1) shows the averages of sister chromatid exchanges obtained after cytological examination of human lymphocytes treated with the different concentration

The data obtained of Cytological examination revealed that sister chromatid exchanges are given in table (1). However the average of SCEs was found to be 2.2 in the control group and it ranged from 2.2 to 14.5 as shown in table (!). there results, however, gave a strong evidence that hibiscus extract and green tea are capable in decreasing Primary DNA damage it seems probable

that these compounds play an important role in repairing damage induced by cyclophosphamide.

Table(2) illusfrates the chromosomal aberrations induced in human lymphocyte cultures. Total aberrant metaphases ranged from 3% to 25% for the negative and positive control, respectively. These data, however, presented the second evidence that Hibiscus extract and green tea was proven to be capable at the line of this study In decreasing the chromosomal aberrations, there data, however, gave the second strong evidence that hibiscus and green tea extract have anticlastogenic activity.

Mitotic index:

Cytological examination showed that the mitotic index (estimated in absence of colcemid) was found to be 14.80 in the control group

Table 1. In vitro induction of SCEs, in human Lymphocytes

Conc.	SCEs X=SE	Range
Control	2.2 ± 0.01	1-3
* Positive control	14.5 ± 1.4	9-18
Hibiscus 10 mg	2.1 ± 0.01	1-3
10 mg +PC	4.2 ± 0.02	2-4
20 mg +PC	6.3 ± 0.01	2-8
40 mg +PC	4.6 ± 0.02	2-6
Green tea 2 mg	2.5 ± 0.01	1-3
2 mg	3.4 ± 0.01	1-5
4 mg	4.2 ± 0.02	2-6
8 mg	2.2 ± 0.01	1-4

* 25 mg / 1000 ml media

Table 2. Chromosomal aberrations in human lymphocytes

Conc.	Deletion	Stickiness	Breaks	RCF	Total aberrant metaphases
Control of	1	2	-	-	3
* Positive control	8	10	3	4	25
Hibiscus 10 mg	-	2	2	-	4
10 mg +PC	2	6	-	1	9
20 mg +PC	2	2	2	1	7
40 mg +PC	4	2	3	1	10
Green tea 2 mg	1	4	1	1	7
2 mg	2	2	1	-	5
4 mg	4	2	-	1	7
8 mg	6	3	1	1	11

* 25 mg / 1000 ml media

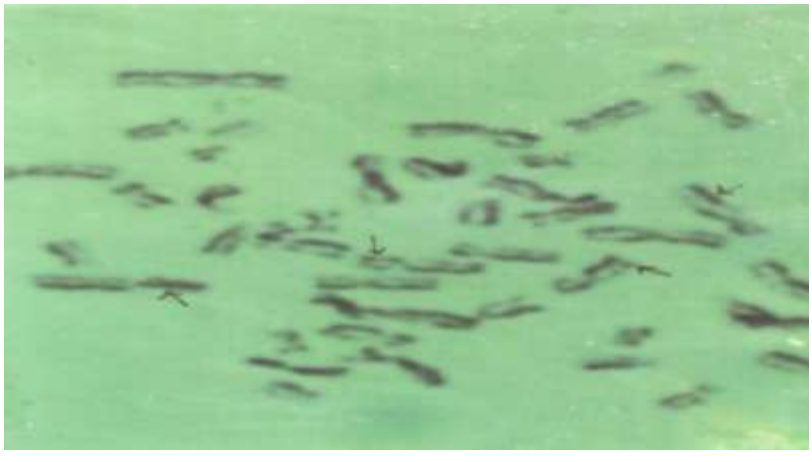

Figure 1. Photomicrograph showing human lymphocyte with (14) SCEs

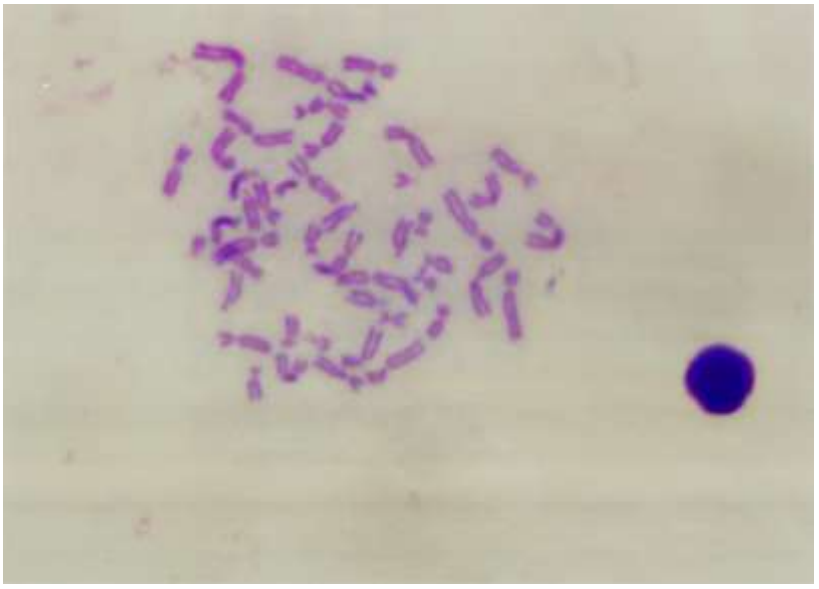

Figure 2. Photomicrograph showing human lymphocyte in negative control

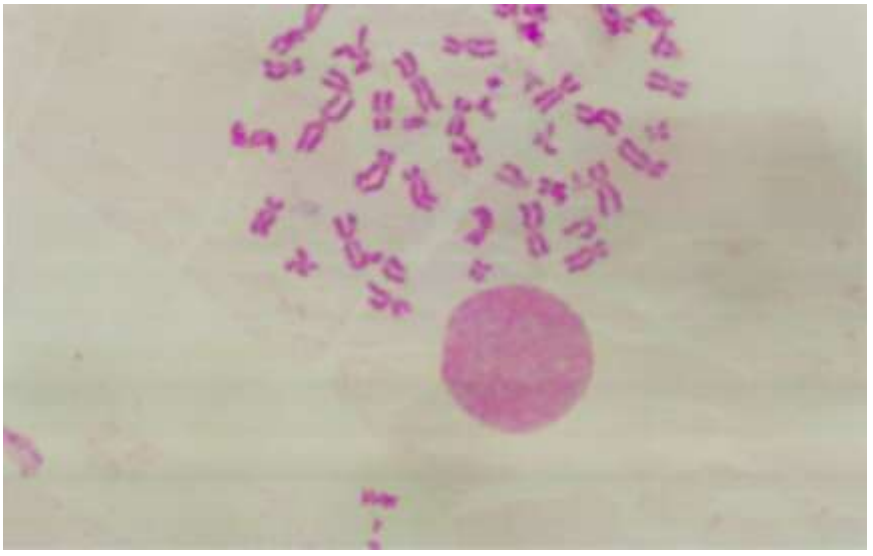

Figure3. photomicrograph showing human lymphocyte with deletion and fragment.

DISCUSSION

One of man's concerns about his surrounding environment is the pollutive impact of various physical and chemical agents released at high levels as a result of recent intensive industrillization. Consequently, scientists designed several techniques in ordrd to monitor, model, and to assess pollutants in an ecosystem. (Seehy et. al.,1989).

Environmental biologists concentrated their efforts to elucidate clearly the potentiality of such pollutants to induce harmful effects on the biological systems. Several tests were recommended for achieving valid results, these tests include battery type tests, where test organisms are of different organization complexity and from groups, i.e., microorganisms, plants, and animals.

Sister chromatid exchanges (SCEs) represent exchange of DNA between replication products at homologous points. At metaphase, these represent symmetrical exchanges between sister chromatids at identical loci, which can only be visualized if the sister chromatids can be distinguished either by radioactive labeling or differential staining following incorporation of 5-bromodeoxyuridine. Although several models for the origin of SCEs have been proposed (e.g., Painter, 1980), the molecular basis of their information has not been elucidated. These may represent some repair processes associated with DNA replication.

Agents which induce chromosomal aberrations in an s-phase may result in metaphase and might be designed as macro DNA damage.

It may be observed that many hazardous factors exist and different foci of attention are considerd initially: acute toxicity, carcinogenesis and teratogenesis are the key points.

Reproductive and genetic toxicity have emerged at present are rapidly developing areas of clinical and basic (animal) research. It should be kept in mind that the contrast between data from experimental studies and observational data in human exemplifies an important distinction in toxicology, that between hazard and risk. Experiments aimed purely at determining whether an agent has the potential to damage a biological system are concerned with hazard. The concept of risk combines hazard and the biological context. It incorporates not only the level of exposure but levels reaching the target tissue, the effects of toxifying\ detoxifying metabolic systems, repair processes, and any other factors modulating the final response. The ultimate objective of toxicological

Agropesticides are of ample world-wide use. Among them, organophosphoric (OP) compounds, though restricted in many countries, are nevertheless employed in numerous places.

Amenable to sensitive and rapid assessment with the technique. Particular features of these studies appear encouraging, however. The studies showed that (1) analysis of SCEs is rapid and not subject to the same spontaneous background variability as conventional chromosome analysis; (2) blood samples could be collected, transported over long distances , and grown in culture under relatively uniform conditions; and (3) effects from low-level occupational exposures can be detected with a degree of sensitivity unequaled by conventional cytogenetic analysis.

Analysis of SCE in human cells may also play a role in establishing the dose received, or at least in identifying affected individuals following accidental exposure to known genotoxic substances. For example, the target site (chromosome) does on a somatic cell basis may be assessed by degree of the increase in SCE in workers located at various distances from the primary release site. This analysis is rapid and sensitive and can be completed very shortly after the actual exposure has occurred (about 2-3 days). Based on this information, workers requiring immediate attention or long- term follow up can be identified. There are probably no other available tests for genetic monitoring which more closely meet the requirements of a human dosimeter than does the sister chromatid exchange technique (Brusick, 1986 and Seehy 2013).

The analysis of mitotic as well as metaphase indices presented evidence that the tested drug was proven to be positive in inducing significant increases in mitotic phases (in absence of colcemid) as well as metaphase index. increases in mitotic index were found to be higher than those in metaphase index. These differences may be caused by the colcemid treatment. However, this result presented an evidence that ''anovlar 1'' enhances the cell proliferation. The analysis of mitotic abnormalities gave an evidence for the capability of indoxan in inducing stickiness;and fragments. Stickiness is a common tybe of chromosomal aberrations due to either alteration of the net charge of chromosomal proteins.., histones or non-histones,or by induction of DNA cross-linking (Emery & Muller, 1988; Hafez, 1998; Seehy, 2003; and seehy, 2013).

The result obtained, however, revealed that the tested compound indoxan was not only effective in inducing in changes chromosome structure but also in inducing spindle apparatus aberration, since ploypoid cells were odserved. Therefore, it could be suggested that indoxan is a potent calstogen upon human genome. Such a conclusion agrees with that found by Khalifa (1992) on mice chromosomes; and with that reported by Ahmed et al (1985) on Salmonella test: and with that reported by Seehy & Hafez (1992) who investigated the clastogenic as well as the nutagenic effects of Nordette and Norminest on yeast, onion, bean, mice; and human lymphocytes. The present results however showed that Hibiscus and green tea, at the level of this work, have anticlatogenic activity upon indoxan effect in mice genome.

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الملخص العربي

النشاط المضاد لتكسير الكروموسومات لمستخلص الكركديه والشاي الأخضر

مُحَّد الصيحي، حسن مصباح، ماجده القاضي وأحمد السيد

يهدف هذا البحث لمعرفة النشاط المضاد لتكسير الكروموسومات المستحدثة بواسطة عقار الاندوكسان وهو السيكلوفوسفاميد والذي يستخدم في العلاج الكيماوي لسرطان ثدي السيدات.

هذا وقد تم توظيف خلايا الدم البيضاء المتحصل عليها من متطوعين أصحاء غير مدخنين وغير متعاطين لعقاير طبية وفي وجود مشابه الثايميدين المسمي بروموديأوكسي يوريدين لدورتين متتاليتين من دورات الانقسام الميتوزي هذا وقد أظهرت نتائج تحليل تبادل الكروماتيدات الشقيقة أن الكنترول الموجب قادر علي انتاج متوسطات من الكروماتيدات الشقيقة بلغت 14,5 بمدي تراوح من 9 – 18 تبادلاً للخلية كما أظهرت نتائج تحليل تبادل الكروماتيدات الشقيقة أن مستخلص الكركدية ذو قدرة علي اصلاح الضرر الاولي للـ DNA حيث أن التركيزات المستخدمة جميعها أثبتت قدرتها علي خفض تبادل الكروماتيدات الشقيقة مما يبرهن علي أن مستخلص الكركديه له قدره علي اصلاح الضرر الأولي الذي يحدثه أو يسببه الاندوكسان

وبالنسبة لمستخلص الشاي الأخضر فقد أوضحت النتائج من تحليل الكروماتيدات الشقيقة ذو قدرة علي اصلاح الضرر الأولي للـ DNA حيث أن التركيزات المستخدمة جميعها أثبتت قدرتها علي خفض تبادل الكروماتيدات الشقيقة مما يبرهن علي أن مستخلص الشاي الأخضر له قدره علي اصلاح الضرر الأولي الذي يحدثه أو يسببه الاندوكسان.

أما بالنسبة لتحليل الشذوذات الكروموسومية أظهرت النتائج أنه في حاله استخدام الاندوكسان كان مجموع الشذوذات الكروموسومية عالياً جداً كما أوضحت النتائج أيضاً أنه في حالة استخدام مستخلص الكركديه كان ذو قدره عاليه علي اصلاح الضرر الأولي الحادث في الـ DNA حيث أن التركيزات المستخدمة جميعها كان لها دور في خفض نسبة الشذوذ الكروموسومي الحادث نتيجة استخدام الاندوكسان.

وبالنسبة لمستخلص الشاي الأخضر أظهرت النتائج أيضاً أن التركيزات المستخدمة جميعها لها قدرة علي خفض الضرر الأولي الحادث في الـ DNA نتيجة استخدام الاندوكسان أيضاً.