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Research Article

EFFECT OF *ACACIA SINUATA* AND *ADENANTHERA PAVONINA* ON CISPLATIN INDUCED GENETIC DAMAGE IN CULTURED HUMAN PERIPHERAL LYMPHOCYTES

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ABSTRACT

Many plants are now been exploited for their medical and pharmacological properties. *Acacia sinuata* has been reported with cure for infectious disease and organ specific disease, confirmed to have analgesic activity and also used to cure jaundice and malarial fever in Ayurveda. Similarly, *Adenanthera pavonina* is reported for treating wounds, boils, rheumatism, arthritis, diarrhea, and even leprosy. The plant has emetic properties and can induce a person to vomit, but literature studies showed that the safety assessment of such widely used medicinal plants has not been done hence they were chosen for the purpose. The plant extracts of *Acacia sinuata* and *Adenanthera pavonina* were tested using chromosomal aberration assay and comet assay for their genotoxicity and further studies were performed to check for the genome repair capability of the plants. The lymphocyte culture was exposed to cisplatin to induce damage to the genome and then treated with the plant extract. In this paper the extracts of A. sinuata and A. pavonina at different concentration significantly increased the mitotic index compared to the cisplatin treated cell alone; this decrease in cell proliferation may be explained by permitting the repair of cisplatin induced DNA damage. Both plants show little genomic activity with increased concentration. All tested concentrations of extract from *A. sinuata* and *A. pavonina* had no genotoxic effect on the human blood in vitro.

Keywords: *Acacia sinuata*, *Adenanthera pavonina*, Cisplatin, Genome repair, Plant extract

INTRODUCTION

Medicinal plants have been used in folk medicine. In recent times, scientific study of their effects has been flourished but some of these can cause adverse effects or may interact with other medications[1]. It is known that, in general, green plants are a primary source of antimutagens as well as natural toxic agents[2] and many plants contain cytotoxic and genotoxic substances resulting from the long-term use of such plants. Plants produce broad spectrum of polyphenolic compounds as secondary metabolites for their survival under stress conditions. Polyphenols have an ability to neutralize free radicals by donating them electrons or hydrogen atoms which may play a vital role in health benefits[3]. In many places of Argentina, there is a rich tradition of using herbal medicine for the treatment of various infectious diseases like[4] inflammations[5], and injuries[6]. Some plant lectins have been found to possess remarkable anticancer properties in *in vivo*, *in vitro* and in human case studies[7] and have also been successfully adopted for alternative cancer therapy[8, 9]. Plant cause cytotoxicity in cancer cells via different mechanisms namely, by inducing apoptosis, autophagy or necrosis and inhibiting cells growth, preferentially binding to cancer cells membrane or their receptors[10]. After this recognition step, lectins either remain on cell surface or be internalized into the cells and are located in different compartments[11]. The biochemical events that occur during apoptosis lead to several typical morphological cell changes. These changes include loss of cell membrane asymmetry and attachment, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation[12]. Plants display anti-inflammatory[13], hypotensive[14], hepatoprotective[15], antiviral[16], antiurolithiasic[17] and antiallergic activities[18]. Studies on the activities of these glycoalkaloids showed that it inhibit the growth of different cancer cell types, e.g. human colon (HT-29, HCT-15), human prostate (LnCaP, PC-3), human breast (T47D, MDA-MB-231, MCF-7), human hepatoma (HepG2, SMMC-7721), human cervical cancer (JTC-26, HeLa), human leukemia (K562), human glioblastoma (M059J, U343, U251), human osteosarcoma (U2OS), and murine melanoma (B16F10)[19,20,21].

Adenanthera pavonina is a deciduous tree commonly used in the traditional medicine to treat inflammation and rheumatism. Pharmacological studies of the plant showed that the seeds of this plant possess anticonvulsant and central nervous system depressant, analgesic, and anti-inflammatory activity[22, 23]. But there are no evidences demonstrating the antinociceptive activity of the leaves of *A. pavonina* which prompted this study to evaluate the effectiveness of ethanol extract of this plant leaves in different nociceptive models and investigate the possible. The purpose of this study was to contribute towards safe use of medicinal plants by means of the evaluation of the possible cytotoxic and/or genotoxic effects of *Acacia* extracts by chromosomal aberration and comet assays respectively.

MATERIALS AND METHODS

Chromosomal Aberration Assay

Peripheral blood samples were collected and add to medium containing (RPMI1640 (5 ml), phyto-haemagglutinin (200µl) and fetal bovine serum (300 µl) and incubated at 37ºC. After 72 h of incubation, cells were treated with colchicine to arrest them at metaphase stage. The cells were swelled in hypotonic solution and fixative containing methanol and then glacial acetic acid was added. The cells were washed and a clear pellet was dropped onto the slide from a great distance of 10cm. The slide was heated so that the chromosomes spread and become visible under the microscope. At last, the chromosomes were stained with giemsa stain.

For Comet Assay

The frosted slides were covered with agarose containing the whole blood. The slides were immersed for 1 hour in lysis buffer solution with 1% Triton-X. Then the slides were placed on a horizontal gel electrophoresis tank. The unit was filled with electrophoresis buffer and slide was placed in alkaline buffer for 20 mins. Denaturation and electrophoresis were performed at 4^0 C under the dim light. Electrophoresis was carried out for 20 mins at 25º C. After electrophoresis the slides were rinsed with neutralization buffer and staining of slides were done with ethidium bromide (Etbr) and then stored at 4º C until analysis.

RESULT AND DISCUSSION

Standardization of the Chromosomal Aberration Assay

The chromosomal aberration assay was standardized for different combinations of the reagents and incubation for better results. The chromosomal aberration was done for positive control (Fig.1). In Positive control only blood sample was taken. Cisplatin being a mutagen causes genome damage by disintegrating the chromosome (Fig.2). These are the chromosomes taken from healthy human sample which were treated with *Acacia sinuata* plant extract (150mg/ml). This shows that there is no cytotoxic effect caused in the sample which can be attributed to the plant extract. The integrated chromosomes suggest the chromosomal repair activity of the *Acacia sinuata* plant extract (Fig.3). This figure shows the chromosomes from healthy human blood sample that were treated with *Adenanthera pavonina* plant extract (150mg/ml). Since there were no disintegrated chromosomes among the healthy chromosomes, it can be suggested that the *Adenanthera pavonina* plant extract doesn't possess cytotoxic effect. The better integrated chromosomal structures in comparison to the chromosomes from the control sample suggest the genome repair activity of the plant extracts (Fig.4). The table shows the comparative study of the cytotoxic effect of different concentrations of *Acacia sinuata* and *Adenanthera pavonina*. The above data is collected by performing chromosomal aberration assay on the blood sample collected from healthy human (control), the blood sample from healthy human and treated with *Acacia sinuata* and by *Adenanthera pavonina*. From this data we can detect that the plant extracts don't cause any further chromosomal damage but instead can possibly hold potential to repair the genetic damage (Table1).

Analysis of the Slides Prepared for Study of the Cytotoxic Effect of the Plant Extract

The slides prepared from lymphocyte culture treated with plant extracts were analyzed under the microscope. The table contains data that were collected by treating healthy human blood samples to corresponding reagents (cisplatin, *Acacia sinuata* plant extract, *Adenanthera pavonina* plant extracts). The lymphocyte culture was made by using the healthy human blood sample and it was then exposed to the various reagents in question at different concentrations to check for their cytotoxic effect and also to see if any effect is present that varies with the concentration. From the data in the table above we can deduce that both plant extracts don't possess any cytotoxic effect. In contrast, the increased number of mitotic cells scored in comparison to the respective controls suggests that they may possess a chromosome repair activity instead (Table 2). Mitotic index is universally accepted measure of assessing chromosomal aberration and cytotoxic effect of any reagent. The data in the table was calculated based on the number of metaphase cells scored as compared to the cells having chromosomal or chromatid level aberration. From the above data, the absence of the cytotoxic effect of the plant extracts can be confirmed. And an increased mitotic index suggest the chromosomal repair activity of the plant extracts with an increase in concentration of plant extracts as compared to controls (Table 3).

Analysis of the Slides to Determine the Mitotic Index for the Study of Genome Repair Activity

The lymphocyte culture pre-exposed to the mutagen (cisplatin) was treated with the plant extract for checking its activity in repairing the DNA damage to the lymphocytes. Table 4 shows that if we increase the concentration of plant extract then genome activity was also increases. The mitotic index of *Acacia sinuata* showed little genomic activity while mitotic index of *Adenanthera pavonina* showed more genomic activity than *Acacia sinuata*. The table shows the data collected by pre-exposing the lymphocyte culture from healthy human blood sample to the mutagen, cisplatin (0.5mg/ml). Cisplatin being a mutagen caused damage to the chromosomes. The pre-exposed samples were then further treated with different concentrations of plant extracts of *Adenanthera pavonina* and *Acacia sinuata* to assess the chromosomes repair activity and to compare it with increasing concentrations. The cells in mitosis were counted under the microscope. From the data collected, it can be suggested that the two plants possess chromosome repair activity (Table 4). The mitotic index is universally accepted measure for assessing chromosomal aberration. The data in the above table were calculated by comparing the number of cells score in mitosis phase to the number of cells containing chromosomal damage or chromatid level damage. The calculated data confirms that both the plant extracts show chromosome repair activity. Also on comparing the chromosome repair activity of both plant extracts, it can be deduced that *Adenanthera pavonina* has higher level of genome repair activity as compared to that of *Acacia sinuata* (Table 5).

Comet Assay to Quantify the DNA Damage of Chromosomal Aberration in each Case

Comet assay was done for negative control which is the lymphocyte culture of the healthy human blood sample. We can observe that the nuclear structure is intact and no tail can be seen. Hence this indicates that no DNA damage is caused in absence of cisplatin (Fig.5). Then the comet assay was done for positive control, which contains lymphocyte culture of the healthy human blood samples exposed to cisplatin. The denatured cell with a trailing tail was observed when nucleus was pre-exposed to cisplatin. This showed that cisplatin has caused damage to the cell nucleus and the trailng tail is actually the fragments of degraded DNA. Hence it can be concluded that cisplatin causes chromosomal degradation and can be assessed using Comet Assay (Fig.6). Now, when these samples were treated with *Acacia sinuata* plant extract, a well-integrated nucleus was observed which confirms that *Acacia sinuate* is not cytotoxic (Fig.7). Similarly, the comet assay was also performed by pre exposing the lymphocyte culture with cisplatin and treated with

Adenanthera pavonina (150 mg/ml) extract and then it was observed that there is no damage done to the nucleus which confirms that *Adenanthera pavonina* is not cytotoxic (Fig.8). The comet assay was also analyzed for lymphocyte culture which was exposed to cisplatin and then treated with plant extracts. From the figure it can be observed that the genetic damage caused was lesser than that compared to the damage caused to the lymphocyte culture cells when exposed with cisplatin alone(fig.9). From the figure it can be observed that the genetic damage caused was lesser than that compared to the damage caused to the Lymphocyte culture cells when exposed with cisplatin alone (Fig.10).

Although plants are very often used in traditional medicines, 70–95 % of the populations in majority of the developing countries rely on traditional medicines for their primary health care but their effect on the genetic material is poorly known in most cases. These plants can be used to treat various diseases such as skin disease, bone disease, jaundice, leprosy and also possess anti-bacterial and analgesic properties. Our results showed that all tested concentrations of extract from *A. sinuata* and *A. pavonina* have no genotoxic effect on the human blood in vitro. Additionally, all tested concentrations of *A. sinuata* and *A. pavonina* showed satisfactory data supporting that the medicinal plants had little genome repair activity. Our results revealed that the extracts of *A. sinuata and A. pavonina* at different concentration significantly increased the mitotic index compared to the cisplatin treated cell alone; this reduction in cell proliferation may be explained by permitting the repair of cisplatin induced DNA damage. *Acacia sinuata* showed a little amount of genome repair activity which increased with an increase in the concentration of the plant extract. *Adenanthera pavonina o*n contrary showed increased genome repair activity compared to *Acacia sinuata* which also increased with increase in concentration. In accordance with the conclusions, plant extracts of *A. sinuata and A. pavonina* reflected the induction of apoptosis in the cells which collected a large quantity of genetic damage induced by cisplatin. This conclusion is confirmed by the fact that in combined treatments of the extract with cisplatin, mitotic index values gradually increases until a statistically significant increase occurs in the treatments with the highest concentrations of the tested extract. Our results confirmed the safe use of *A. sinuata* and *A. pavonina* extract in the tested concentrations and its important function in prevention from induced genetic damage. In the cancer therapy, these extract may prevent genotoxic effects.

Table 1: Chromosomal aberrations analysed from lymphocyte culture treated with plant extracts

		Control	*Acacia sinuate*			*Adenanthera pavonina*		
Types of aberration		10	75 ug/ml	150 ug/ml	300 ug/ml	75 ug/ml	150 ug/ml	300 ug/ml
	No. of cells scored	300	300	300	300	300	300	300
Chromosome	Di-frag	4	3	3	2	4	3	2
	Ac-frag	4	2	1	1	3	3	1
	Ac-rings	0	1	0	0	0	0	0
Chromatid	Gaps	9	8	6	5	6	6	4
	Breaks	13	11	11	9	10	9	9
	Exchanges	2	0	0	1	0	2	0

Table 2: **Cytotoxic effect on lymphocyte culture treated with plant extracts and cisplatin**

Treatments	**Concentration**	**No. of cells scored**	**No. of Cells in Mitosis**
Control (-ve)	-	4600	56
Control (+ve)	0.5mg/ml (cisplatin)	4200	21
Acacia sinuata	75 µg/ml	5200	60
	150µg/ml	4900	68
	300µg/ml	5000	74
*Adenanthera Pavonin*a	75 µg/ml	5300	66
	150µg/ml	5100	72
	300µg/ml	4500	74

Table 3: Mitotic index data of lymphocyte culture treated with plant extracts and cisplatin

Treatments		No. of Metaphases scored	No. of cells with chromosome- type aberrations	No. of cells with chromatid- type aberrations	Mitotic Index
Control (-ve)		56	23	20	1.21
Control (+ve) 0.5mg/ml (Cisplatin)		21	11	06	0.50
Acacia sinuate	75 µg/ml	60	19	21	1.15
	150µg/ml	68	20	18	1.22
	300µg/ml	74	17	19	1.48
Adenanthera pavonina	75 µg/ml	66	24	26	1.24
	150µg/ml	72	22	24	1.41
	300µg/ml	74	18	22	1.64

Table 4: Analysis of the data from the slides prepared from lymphocyte culture pre-exposed with mutagen (cisplatin) and then treated with plant extracts

Treatments	Concentration Plant+cisplatin	No. of cells Scored	No. of Cells in Mitosis
Control (-ve)	-	4600	56
Control (+ve)	0.5mg cisplatin	4200	21
Acacia sinuate	75 µg/ml +0.5mg/ml	4500	24
	150µg/ml+0.5mg/ml	4300	27
	300µg/ml+0.5mg/ml	4700	31
*Adenanthera pavonin*a	75 µg/ml+0.5mg/ml	4500	27
	150µg/ml+0.5mg/ml	4600	31
	300µg/ml+0.5mg/ml	4800	36

Table 5: Mitotic index data from the lymphocyte culture pre-exposed to mutagen (cisplatin) and treated with plant extracts

Treatments		No. of Metaphases scored	No. of cells with chromosome-type aberrations	No. of cells with chromatid- type aberrations	Mitotic Index
Control (-ve)		56	2	5	1.21
Control(+ve) : 0.5mg Cisplatin		50	11	13	0.50
Acacia sinuate	75 µg/ml+0.5mg/ml cisplatin	154	5	8	0.53
	150µg/ml0.5mg/ml cisplatin	158	7	7	0.62
	300µg/ml0.5mg/ml cisplatin	158	4	3	0.65
Adenanthera pavonina	75 µg/ml0.5mg/ml cisplatin	152	2	9	0.60
	150µg/ml0.5mg/ml cisplatin	151	3	5	0.67
	300µg/ml0.5mg/ml cisplatin	157	5	4	0.75

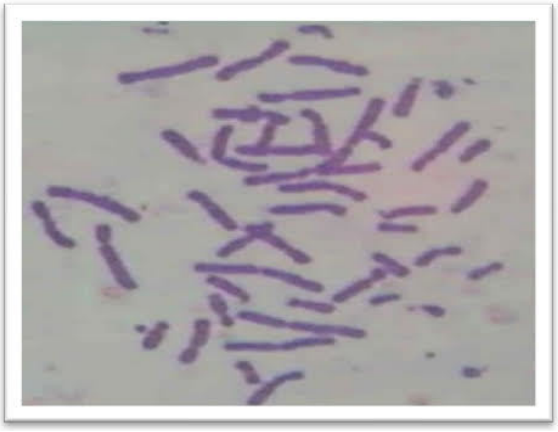

Fig 1: Metaphase Chromosomes (Control)

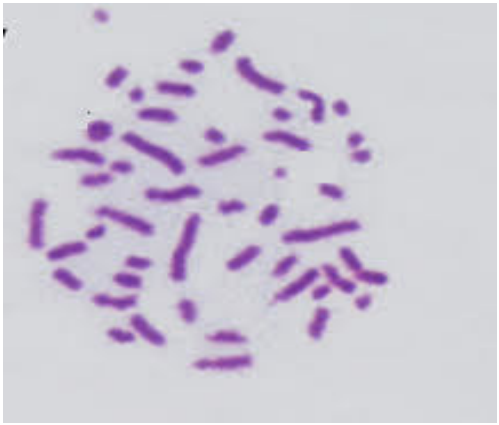

Fig 2: Metaphase Chromosomes treated with *Acacia sinuate*

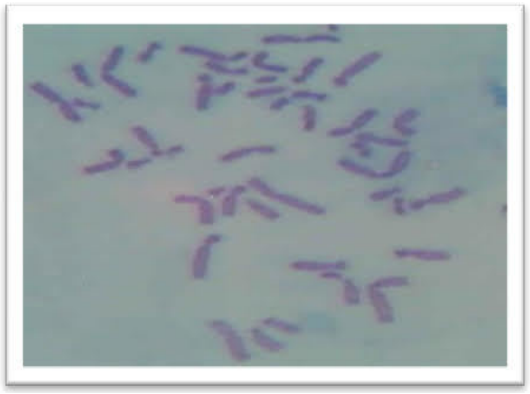

Fig 3: Metaphase Chromosomes treated with *Adenanthera pavonina*

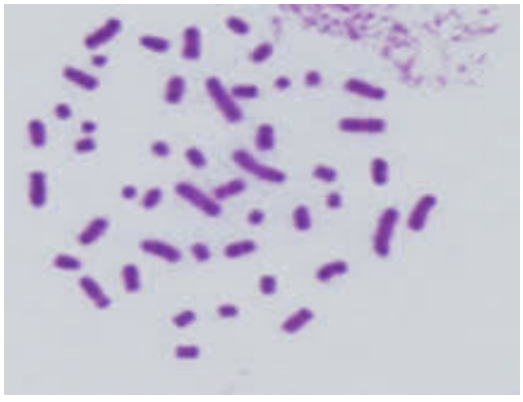

Fig 4: Metaphase Chromosomes exposed to Mutagen (Cisplatin)

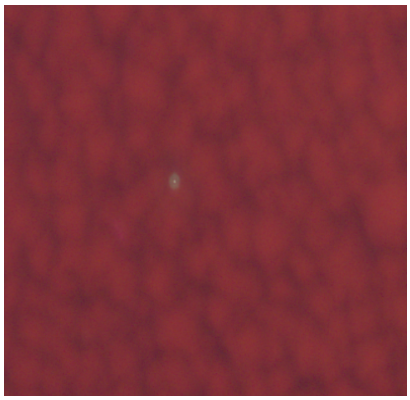

Fig 5: Control cell (In absence of Cisplatin)

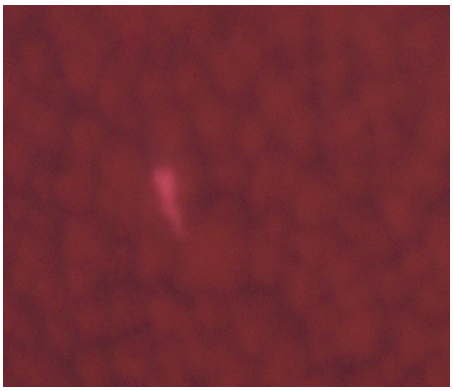

Fig 6: Control cell (+ve Cisplatin)

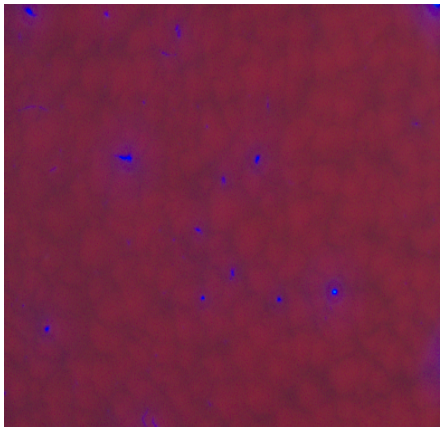

Fig 7: *Acacia sinuata* treated cells (Cytotoxic studies)

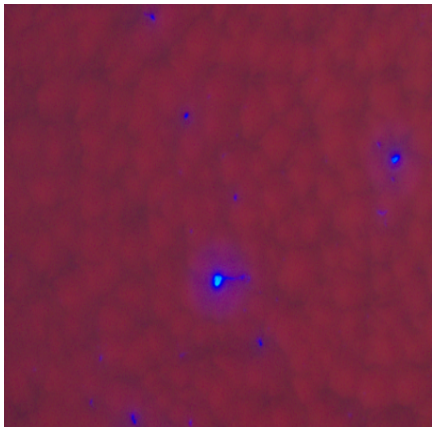

Fig 8: *Adenanthera pavonina* treated cells (Cytotoxic studies)

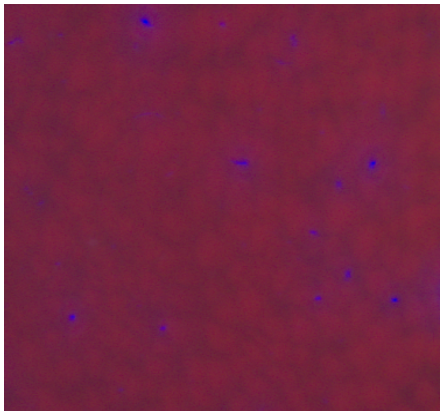

Fig 9: *Acacia sinuata* treated cells (Genome repair studies)

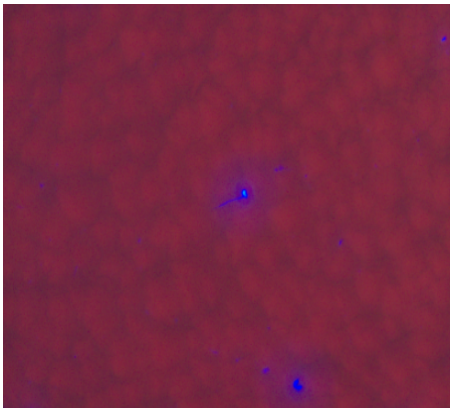

Fig 10: *Adenanthera pavonina* treated cells (Genome repair studies)

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