ANTIBACTERIAL AND CYTOGENETIC EFFECT OF *Cassia italic*a LEAF EXTRACT ON ALBINO MALE MICE

Ebtehal H. Al-Naimy[*], Maythem M. Al-Amery[**] and Ruqia M. Al-Azy[***]

[*] Biotechnology Research Center, Al-Nahrain University.

[**]Biology Department, College of Science for Women, Baghdad University.

[***] Biotechnology Department, College of Science, Al-Nahrain University.

Abstract

This study was designed to evaluate the antibacterial and cytogenetic effect of *Cassia italica* (Senna) on metaphase index and micronucleous formation in polychromatic erythrocytes of bone marrow cells of mice. Results showed that *Cassia italica* leaves methanolic extract caused a significant inhibition in growth of *E.coli* and *Staphylococcus auerous* and *Candida albicans* especially at high concentrations in comparison with the negative control (Distilled water) and positive controls (Tetracycline). On the other hand the results showed that *Cassia italica* extract caused a significant increase in metaphase index and a significant decrease in micronucleous formation in bone marrow of mice in comparison with the negative control (Distilled water) and positive controls (Cyclophosphomide).

Introduction

Finding the healing power in plants is an ancient idea. People on all continents have long used hundreds if not thousands, of indigenous plants as remedy dating back to prehistory. There is an evidence that Neanderthals living 60,000 years ago in present-day Iraq used plants such as hollyhock, [1, 2]. These plants are still widely used in ethno- medicine around the world. Historically, therapeutic results have been mixed quite often cures or symptom relief resulted, but poisonings occurred at a high rate, as well. Currently from one-quarter to one-half of all pharmaceuticals dispensed in the United States have high-plant origins and very few are intended for use as antimicrobials, because we have relied on bacterial and fungal sources for these activities. Since the advent of antibiotics in the 1950s, the use of plant derivatives as antimicrobials has been virtually nonexistent.

Senna plant (***Cassia italica***) is considered as a medicinal plant and its grown in different parts of Iraq [3]. Senna plant was described by [4] who found that biologically active compounds (Coumarins, carotenoids, flavonoids, anthraquinones, tannins, sugars). While [5] found that anthraquiones extract from senna leaf have a significant inhibitory effect (bacteriostatic) on three types of bacteria like, *Bacillus anthraces* and lethal effect (bactericidal) on *Pseudomonus aeruginosa*, another study showed that the methanol extract of senna plant was anti-inflammatory, anti-pruritic and analgesic [6] on lab rat. Besides another chemical compound was found in senna plant (Resveratrol) as an anticancer and had the ability to inhibit cancer by arresting the cell cycle through inhibiting (Cdk1), Cdk2, P53 and Cyclins so that the cell undergoes Apoptosis, [4,5]. Chemotherapy is the primary therapy to cure a small percentage of malignancies, it was used as adjuvant therapy to decrease the rate of relapse or improve the disease-free interval to palliate symptoms. The therapy also aimed to prolong survival in some cases of incurable malignancies prior to surgery to reduce the size of tumor, rendering it more operable post surgery to decrease the risk of occult micromestaseses of tumor stem cells outside the primary field, [7]. Mitotic index assay is defined as the ratio of the number of cells in a population undergoing mitosis to the total number of cells, [8]. Therefore, by the employment of this assay, the effect of different physical and chemical agents on the mitotic response can be detected, and studies have revealed that the mitotic index can be affected negatively or positively by chemicals, radiations, drugs and medicinal plants [9, 10]. Micronucleus are cytoplasmic chromatin–containing bodies formed when a centric chromosome fragments

or chromosomes lag during anaphase and fail to become incorporated into daughter cell nuclei during cell division. Because genetic damage that results in chromosome breaks cause structurally abnormal chromosomes, or spindle abnormalities leads to micronucleus formation. The incidence of micronuclei serves as an index of these types of damage, [11].

This study aims to evaluate the antimicrobial and cytogenetic effect of alcohol extract of senna on metaphase index and micronucleus formation on bone marrow cells of lab rats.

Materials and Methods

Sample collection

A quantity of (250) g of senna plant leaves were collected from the garden of the college of science-Baghdad university. The leaves were cleaned, dried and put in the shade at room temperature, then grounded and prepared for extraction.

Methanolic extraction

A quantity of 30 g of grounded plant leaves were weighed and put in thumble tube in soxhlet apparatus. Aliquots of 200 ml from methanol alcohol were used for extraction at 45 ^{o}C for7 hours, after that the solvent was evaporated by exposing the extraction to air then the residue was put in an incubator (37 ^{o}C) until the solvent totally evaporated. The extraction was collected and weighed then diluted with distilled water to prepare 20% weight /volume, then the extract was sterilized with Millipore filter paper (0.22 μm**),** [12].

Microbial isolates

Eschereshia coli, Staphyloccocus aureus, and Candida albicans were obtained from the microbiology Lab., Biotechnology Research Center, Al-Nahrian University where they were already diagnosed.

The approximate bacterial colonies where collected by using serial dilutions to have $10^{8}\times1$ CFU/ml.

Laboratory Animals

Albino male mice (swiss) were used in this study. They were obtained from Biotechnology Research Center (Al-Nahrain University). Their age ranged between 8-9 weeks, and their weight was 23-27 grams at the beginning of the experiments. They were caged in the animal house of the supplier, in which the temperature was 23-26 ^{o}C, and a light: dark periods of 10:14 hours/day. The animals had free excess to diet (standard pellets) and drinking water during all experiments.

Experimental Design

First Stage

Three concentrations (50, 100, 150 mg/ml) of methanol extract of senna leaves were prepared to investigate their inhibitory effect against *E.coli*, *S.aurous* and *C. albicans*.

- **Preparation of microbial isolates**

The bacterial and yeast suspensions were prepared by inoculating bacterial and yeast culture in test tubes containing 10ml of NB for bacteria and PDB for yeast respectively, after 18 hours of incubation at 37 ^{o}C, duplicate serial dilution were prepared for culture and read with Spectrophotometer at wave length 420 nm.

- **Inhibitory effect of methanolic extract against tested microorganism**

Agar-Well Diffusion method was used to detect the inhibitory effect of plant extract against bacteria and yeast, by making 6mm diameter wells using pasture pipette on the agar media, then 0.2 ml of the each concentration (50, 100 and 150 mg/ml) of plant extract were put in each well, except one filled with distilled water as a negative control then 0.1 ml of bacteria and yeast suspension was added to each well and kept in a refrigerator for 24 hours for diffusion, after incubation at 37 ^{o}C for 24 hrs where the inhibitory effect of plant extract was determined by measuring the diameter of inhibition zone around each well**,** [13].

- **Detection of minimum inhibitory concentration for plant extract against tested microorganism:**

Serial dilutions (25, 50, 100 and 150 mg/ml) were prepared from the final concentration of plant extract 150 mg/ml using Nutrient broth. Then 0.1 ml of each bacterial suspension was transferred to each dilutions of plant extract, after incubation for 24 hrs at 37 ^{o}C. Results were read by spectrophotometer at 240 nm and compared with control tube 1 and 2 compared with the control tube : 1 containing N. broth with bacteria only and

control tube 2: containing N. broth and plant extract without bacteria, [14].

- **Sensitivity of bacterial and yeast isolates against Tetracycline**

Antibiotics sensitivity test of tetracycline supplied by Samara pharmaceutical Co. against bacterial and yeast isolates were done using the agar-Well Diffusion method. Serial dilutions (50, 100, 150) mg/ml of the antibiotic were made and 0.02 ml of each concentration was introduced into each well on the agar medium streaking previously with tested bacteria and yeast and allowed to stand on the bench for about one hour for proper diffusion. Then incubated at 37 ^{o}C for 24 hrs. The resulted inhibition zones obtained were measured in millimeters and compared against the corresponding concentrations of plant extract**,** [14].

Second Stage

In this stage, the cytogenetic effects on mitotic index and micronucleus of two doses of plant extract and cyclophosphomide were investigated. The animals were divided into three groups:

- **Group I**: dosed with distilled water (negative controls = 8 animals).
- **Group II**: dosed with cyclophosphomide at a dose of 15 mg/kg (positive controls=8 animals)
- **Group III**: dosed with two doses of the senna plant extract (50 and 100 mg/kg 16 animals).

The plant extract were given orally as a single dose (0.1 ml) per a day for 7 days. Then the mice were sacrificed in day 8 for laboratory assessments. The total number of mice in this stage was 32 animals.

- **Mitotic index in mice Bone marrow cells**

The metaphase index was assessed on somatic cells obtained from the bone marrow of experimental animal mice, according to a pre-established method, [15].

- **Micronucleus test in mice bone marrow cells**

The experiment was done according to [16].

Statistical analysis

The experiment designed using completely random design (CRD) with three replicates. Duncan multiple range test was used to find differences between treatments and estimate the significant differences between groups at (0.05) level of probability, [17].

Results

Antibacterial Effects

The plant extract showed a significant inhibitory effect on bacteria and yeast growth as shown in table 1 plant extract (150 mg/ml) caused a significant growth inhibition than other concentrations on *E.coli* and *S. aurous* reaching 20.25 ± 0.250 and 26.25 ± 0.250, respectively while the concentration (100 mg/ml) caused a significant growth inhibition to *Candida albicans* reaching 15.18 ± 0.350 compared with the negative control (distilled water). On the other hand the plant extract exhibited a significant inhibitory effect on *E.coli and S.aurous* as compared with the positive control which is (Tetracycline Table (2)).

Table (1)

The inhibition zone diameters caused by methanol leaf extracts of Cassia italica on tested bacteria and yeast isolates (Mean ± SE).

Con. / *Bacteria and yeast*	*Mean ± SE*			
	Control	***50 mg/ml***	***100 mg/ml***	***150 mg/ml***
E.coli	6.00 ± 0.00 a	*15.35±0.35 b	16.75±0.250 c	20.25±0.250 d
S. aurous	6.00 ± 0.00 a	21.35±0.350 b	22.75±0.250 c	26.25±0.250 d
C. albicans	6.00 ± 0.00 a	14.35±0.350 b	17.90±0.100 c	15.18±0.350 c

***Inhibition zone + well diameter 6 mm.**

**** Different letters with the same column are significantly different (P ≤ 0.05) between means.***

Table (2)
The inhibition zone diameters caused by tetracycline on tested bacteria and yeast isolates (Mean ± SE).

Con. / Bacteria And yeast	Mean ± SE		
	50 mg/ml	100 mg/ml	150 mg/ml
E.coli	12.75±0.250* a	14.35±0.350 a	15.50±1.500 a
S*. aurous*	14.25±0.250 a	15.15±0.150 a	16.75±0.250 b
C. albicans	13.25±0.250 a	14.25±0.250 a	16.40±0.400 b

**** Inhibition zone + well diameter 6mm.***
***** Different letters with the same column are significantly different (P ≤ 0.05) between means.***

Cytogenetic Study
Mitotic index of Bone marrow

In the present study the metaphase index for bone marrow cells was estimated in which only cells at metaphase were scored in sample of 1000 cells (Fig.(1)), while their percentage was given. A treatment with cyclophosphomide caused a significant reduction in the metaphase index (1.58 %) as compared to the negative control (4.36 %). In contrast, the differences were significant for all doses of plant extract as compared to the negative control (5.26, 6.00 *vs.* 4.36%) as shown in Table (3).

Micronucleus in Bone marrow

Table (4) shows that micronucleus frequencies of poly chromatic erythrocytes from negative control mice (0.80%). The percentage increased to 2.33 % after treatment with 15 mg/kg Cyclophosph-omide drug. This result is significantly different from the negative control (Fig.(2)).

First and second dose causes a significant decrease in micronucleus formation (Table (4)) compared with negative and positive controls.

Table (3)
Mitotic index of bone marrow cells (mean ± SE) of albino male mice treated with senna methanol leaf extracts.

Groups		Dose (mg/kg)	Mean ± SE
Positive Control (Cyclophosphomide Drug)		15	1.58 ± 0.02 a
Negative Control (Distilled Water)		0.00	4.36 ± 0.11 b
Concentration	First dose	50	5.26 ± 0.23 b
	Second dose	100	6.00 ± 1.83 b

**** Different letters with the same column are significantly different (P ≤ 0.05) between means.***

Table (4)
Micronucleus cells of albino male mice treated with Cassia italica plant extract (mean ± SE).

Groups		*Dose (mg/kg)*	*Mean ± SE*
Positive Control(Cyclophosphomide Drug)		15	2.33 ± 0. 6 a
Negative Control(Distil Water)		0.00	0.80 ± 0.05 b
Concentration	First dose	50	0.400 ± 0.100 c
	Second dose	100	0.200 ± 0.100 d

****Different letters with the same column are significantly different (P ≤ 0.05) between means.***

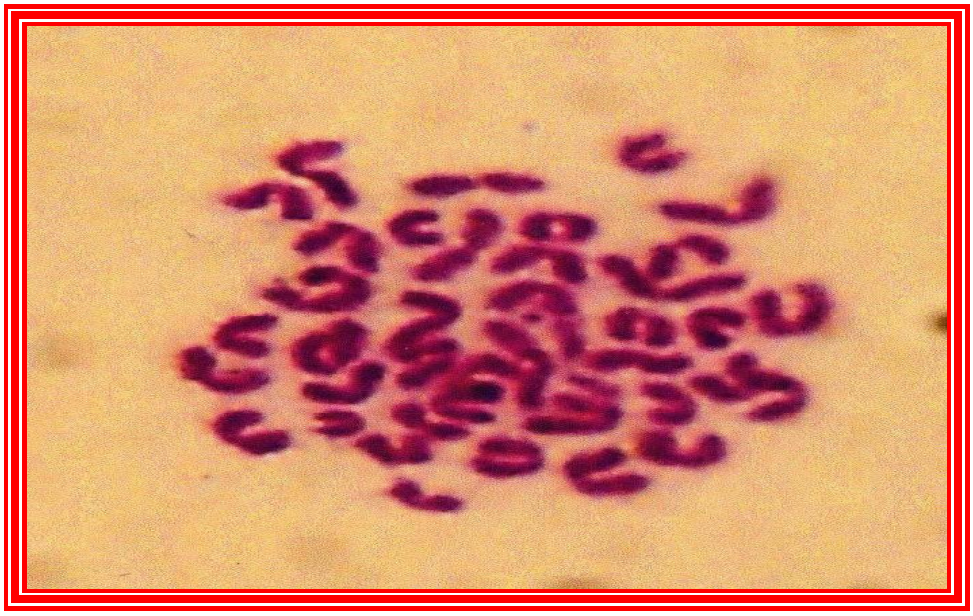

Fig.(1) :A normal metaphase preparation from bone marrow of mouse treated with Cassia italica extract at a concentration of 50, 100 mg/kg after 7 days of treatment (100X).

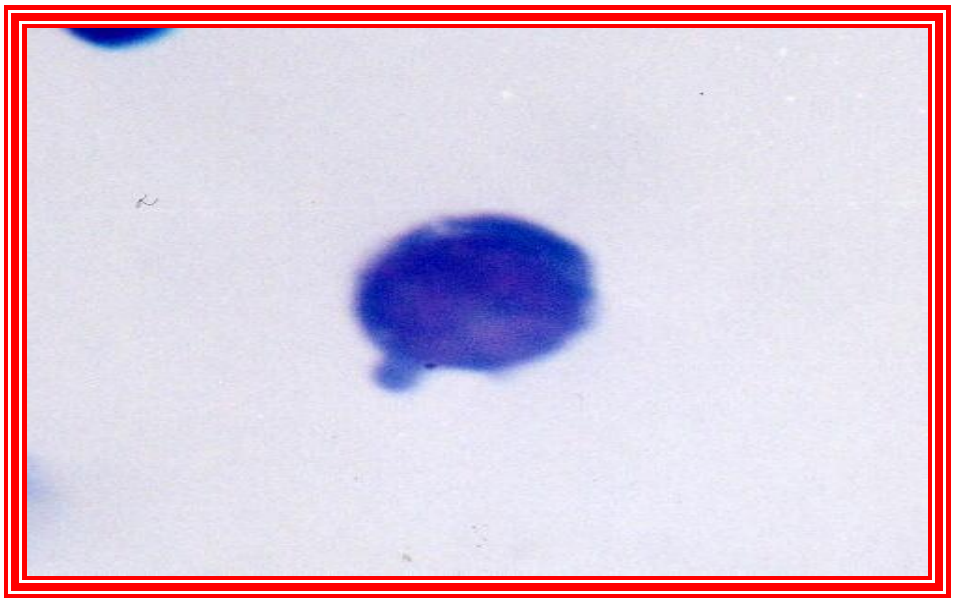

Fig.(2) : Cytogenetic effect in mice treated with cyclophosphomide at a concentration 15mg/kg showing micronucleated bone marrow cell (100 X).

Discussion

The high inhibitory effect of methanol extract of ***Cassia italica*** may be due to the ability of alcohol to extract all or most of active compounds found in plant leaves including glycosides, tannins, cumarins and flavonoids especially rutin and quercetin which represent one of the most abundant natural flavonoids. This result agrees with [18] who found that methanol extraction is a good method to obtain active components from *Cassia italica* leaves especially flavonoids.

The major role of flavonoids on microorganism and yeast is by forming complexes with cell proteins and dissolved proteins that will interact with bacteria cell wall affecting its osmosis, and eventually leading to cell death. It will integrate with bacterial DNA and negatively influencing its vital activities, [19, 20, 21].

The bone marrow metaphase index was investigated on bone marrow lymphocytes, the lymphocyte are divided into T-lymphocyte, which are involved in the cellular immune response and B-lymphocyte which are the main arm of humeral immune response, [22]. When these lymphocytes where treated with two doses of methanol plant extract showed a significant increase in metaphase index (MI), [23]. This may be caused by the presence of mitogenatic agents in the plant extract that induce cell division, [24].

Regarding the micronucleus variation or changes there was a reduction in frequency of MN changes after treating these cells with ***Cassia italica*** extract which may be du to the presence of many active constituents rutine and qurecetin which have an anticancer activity as well as protecting DNA from damage [25], it may be also caused by its ant mutagenic and detoxification activities, [30].

The effect of Cyclophosphomide drug on the metaphase index and micronucleus changes was a reduction in metaphase index of the bone marrow cells. This reduction effect of the drug on bone marrow metaphase index was due to its fatal effect on bone marrow cells [26], or it may be resulted from defect occurring in the mitotic spindle composition during cell division [27] consequently causing mitotic delay, [28].

The micronucleus changes induced by Cyclophosphomide caused chromo-somal damage or damage to the mitotic apparatus of the cells, [11].

Cyclophosphomide is thought to cause breakage of Anaphase Bridge during cell division or damage to DNA [29], therefore abnormality in nuclear shape may be regarded as an indicator of genetic instability, [30].

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الخلاصة

تم في هذه الدراسة التحري عن تاثير مستخلص نبات السنامكي ***Cassia italic*a** على بعض الاحياء المجهرية وفحص التاثيرات الوراثية الخلوية من خلال قياس معامل الانقسام الخلوي الاستوائي وتكوين النوى الصغيرة في خلايا نقي العظم للفئران البيض.اظهرت النتائج ان مستخلص نبات السنامكي سبب بانخفاض معنوي في نموبكتريا*E.coli* وبكتريا *S.aurous* وفي نمو خميرة *Candida albicans* وخاصة عند التراكيز العالية بالمقارنة مع السيطرة السالبة (الماء المقطر) والسيطرة الموجبة (عقار التتراسايكلين). من ناحية اخرى اظهرت النتائج ان مستخلص نبات السنامكي سبب بزيادة معنوية في معامل الانقسام الخلوي وانخفاض معنوي في معدل تكوين النوى الصغيرة لخلايا نقي العظم للفأران بالمقارنة مع السيطرة السالبة (الماء المقطر) والسيطرة الموجبة (عقار السايكلوفوسفامايد). وسبب الاخيرانخفاض معنوي في معامل الانقسام الخلوي وزيادة معنوية في معدل تكوين النوى الصغيرة في خلايا نقي العظم في الفئران.