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Studies on Cytotoxic and Genotoxic potential of Ethanolic extract of *Lawsonia inermis* leaves

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Abstract*---Lawsonia inermis*** is widely known as henna and belongs to Lythraceae family. It contains various phytochemica constituents in every part of plant and shows various pharmacological properties. Secondary metabolites are acts as antioxidants and these are very high in this plant species. In this research studies, ethanolic extract of *Lawsonia inermis* was performed in the blood sample culture for cytotoxicity and genotoxicity effects. The both of the effects of plant extracts were evaluated through measurement of mitotic index (MI), sister chromatid exchanges (SCE) and cell proliferation kinetics (CPK). The blood sample culture was treated with control and various concentrations of extracts (500µg/ml and 1000µg/ml). The mean of MI, SCE and CPK for control were found to be 4.51, 5.19 and 0.52 respectively where as for 500µg/ml of *Lawsonia inermis* extracts are

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4.33, 4.87 and 0.50 respectively and for 1000 µg/ml extracts are 4.18, 4.68 and 0.48 respectively. From the results, we can conclude that the MI and frequency of SCES of ethanolic plant extracts are remarkably similar to that of control which indicates that the *Lawsonia inermis* plant extracts have no substantial cytotoxicity and genotoxicity effects in cultured blood lymphocytes.

Keywords---*Lawsonia inermis*, Cytotoxicity, Genotoxicity, Miotic index, Blood sample.

Introduction

Herbal medicines have been employed in traditional medicine. One kind of dietary supplement is herbal medicine.[1,2] Herbal remedies are used by people to try to maintain their health. The knowledge of the creation of new ideas associated to the use of medicinal plants and also the evolution of consciousness has enhanced. The potentiality of pharmacists to address the challenges that have arisen with the expansion of professional services in the development and implementation of human life. *Lawsonia inermis* is widely known as henna and belongs to Lythraceae family.[3] It grows mainly in tropical climates and is used across the world and native to Asia and Africa. It is used as a cosmetic dye.[4] Leaves, flowers, root and stem are used in drug preparation for treatment of various disorders. It acts as a colouring agent. This flowers of plant is used in perfume industry. Lawsone, coumarins, β-sitosterol, napthoquinone[5], tannin and gallic acid compounds are present in this plant species. It shows immunomodulatory effect, anti-inflammatory activity, antioxidant effect, cytotoxic activity, antimicrobial activity, antiparasitic activity and wound healing effects. Cytotoxicity is measured by the mitotic index (MI). Cell population develops as cells and progressing through interphase[6] and mitosis to complete the growth of cell cycle. Many cells lose their ability to divide as they grow and sometimes only divide infrequently. Some cells have the potential to divide rapidly. Cytotoxicity is a characteristic of being harmful to cells.[7] MI is a measure for proliferative condition of population cell. The number of cells in mitosis divided by the total number of cells is known as the mitotic index (MI). It can be calculated from a slide with help of light microscopy. Genotoxicity is measured by Sister Chromatid Exchange[8] (SCE) Test. This test evaluated the potentiality of chemical to stimulate the deoxyribonucleic acid exchange between two sister chromatids of a replicating chromosome. This test is usually performed on human blood lymphocytes.

Materials and Methods

Collection of plant material

The leaves of *Lawsonia inermis* were collected from Visakhapatnam city, Andhra Pradesh State, India. Leaves are cleaned with water and dried under sunlight. Converted in to fine powder and sieved by 120 mesh.

Plant Extraction

10g of powder was mixed with 500ml of ethanol solvent. Continuous extraction was carried out for 10 hours by using the Soxhlet apparatus.[9] After this process, the extract was filtered and ethanol was evaporated by rotary vacuum apparatus. Finally dry extract is obtained and preserved at 5^{o}C temperature.

Blood Sample Collection

Three fresh samples were collected from the participants and deposited in a sterile tube.

Initiation of Blood culture

100µl of mitogen phytohemagglutinin (PHA) was added to 0.5ml of blood sample (BS) are cultured in Roswell Park Memorial Institute (RPMI) 1640 Medium.[10] Two various doses of plant extracts (500µg/ml and 1000µg/ml) were added. 0.1µg of Mitomycin-c was added for mutagen culture. Bromo-deoxy-uridine was added after 24hr. Finally Blood cultures were incubated for 72 hours at 37°C.

Harvesting process of Blood culture

By managing colchicine can inhibit the cell cycle at this stage leaving the gene chromosomes. Colchicine inhibits the growth of microtubules, which are required for the spindle fibres to split the chromosomal genes.[11] To inhibit the cell growth division at metaphase, first 0.1 ml of colchicine (colcimed) was added to blood cultures and incubated for 45min. Then centrifuged at 1000 rpm for 15 min. Again 6ml of potassium chloride (0.75M) was added and incubated for 10min and centrifugation was done with same readings.

Culture Fixations

5ml fixative solution (3:1 ratio of CH_3OH and CH_3COOH) was added to the pellet and centrifuged. Finally we collected tiny pellet and placed on microscopic slides.

Metaphase chromosome spreads preparation

The Chromosome spreads were made by slightly squeezing the cell suspension onto a clear grease-free frozen slide and air-drying it.

Determination of Mitotic index (MI)

The slides were stained with Leishman's stain (10%) in phosphate buffer saline (at pH 6.8) for 10min. Leishman's stain is used in the microscopy for stain blood smears). Whenever the slides were dried and fixed then finally chromosomes were observed. Each blood culture was used to make slides and thousand numbers of lymphocytes[12] (White blood cells) were identifying per culture to measure the MI. It is determine for cell population's proliferative state. It is defined as the number of cells in mitosis divided by the total number of cells. It can be calculated as: MI= Number of cells in mitosis / Total No. of cells X 100.

Determination of Cell Proliferation Kinetics (CPK)

M_i, M_{ii} and M_{iii} metaphases were used to determine cell cycle variations. M_i metaphase chromosomes are substituted to bromo-deoxy-uridine unifiberly in both of chromatids.[13] M_{ii} metaphase chromosomes had bifiberly substitution of bromo-deoxy-uridine in one chromatid. As a result, the cells were mildly stained, revealing the normal SCE pattern. Bifiber integration of bromo-deoxy-uridine in 3rd cell cycle producing mild staining of two chromatids. Such these were identified as M_{iii} metaphases. It can be calculated as : $CPK= M_i +2M_{ii} +3M_{iii} / 100$.

Determination of Sister Chromatid Exchange assay

After 72 hours of slide preparation were used for SCE assay. A droplet of Hoechst 3325 liquid was kept on the slide and set with coverslip for 15 to 20 min. First slides are washed with water and put in the dark chamber place. These dry slides are kept in petridish plates containing 2X Saline Sodiun Citrate buffer solution[14] for 20min and reveal to ultra violet light for 30min. Again slides are washed and dried for 10min. The slides are kept in 2X Saline Sodiun Citrate buffer solutions for two hours at 57°C temperature.[15] The slides are stained by 1% giemsa stain with phosphate buffer. The SCE was used to measure if *Lawsonia inermis* extracts produced any genotoxicity. The preparation of slides are made from each blood culture and number of SCE in the centromere are identified in thousand number of M_{ii} metaphase chromosomes. It can be calculated as:
SCE= Total No. of SCE / Total No. of metaphases chromosomes observed with SCE X 100.

Results and Discussion
Mitotic Index (MI)

Two types of dividing cells are observed on the slides. There are prophase and metaphase stages. The number of dividing and non dividing cells are identified on the slides for the determination of cytotoxicity[16] of control and plant extracts using blood samples (BS) and and shown in table 1 and figure 1.

Table 1
MI of Control and *Lawsonia inermis* extracts using blood samples (BS)

Types of Culture	Control			500 µg/ml of plant extract			1000 µg/ml of plant extract		
	BS-1	BS-2	BS-3	BS-1	BS-2	BS-3	BS-1	BS-2	BS-3
Number of Prophases	02	03	04	03	04	05	05	08	06
Number of Metaphases	46	48	45	44	43	37	38	37	33
Number of dividing cells	49	52	50	48	48	43	44	46	40
Number of cells observed	1027	1167	1056	1045	1113	1044	1002	1104	1001
Mitotic index (MI)	4.77	4.45	4.32	4.59	4.31	4.11	4.39	4.16	3.99
Mean (MI)	4.51			4.33			4.18		

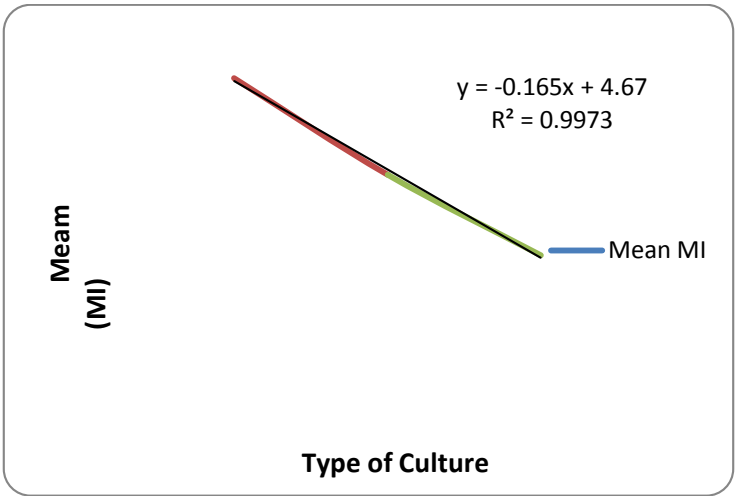

Fig.1. Total Mean values of Mitotic Index (MI) of Control and *Lawsonia inermis* extracts

Sister Chromatid Exchanges (SCEs)

Each blood culture was used to make slides and thousand numbers of metaphase spreads[17] were identified per culture to measure the genotoxicity of control and plant extracts using blood samples (BS) and shown in table 2 and figure 2.

Table 2
SCE of Control and *Lawsonia inermis* extracts using blood samples (BS)

Types of Culture	Control			500 µg/ml of plant extract			1000 µg/ml of plant extract		
	BS-1	BS-2	BS-3	BS-1	BS-2	BS-3	BS-1	BS-2	BS-3
Total no of sister chromatid exchanges observed	5247	4874	5487	4934	4473	5238	4875	4238	4951
No. of metaphases observed	1000	1000	1000	1000	1000	1000	1000	1000	1000
SCE	5.24	4.87	5.48	4.93	4.47	5.23	4.87	4.23	4.95
Mean (SCE)	5.19			4.87			4.68		

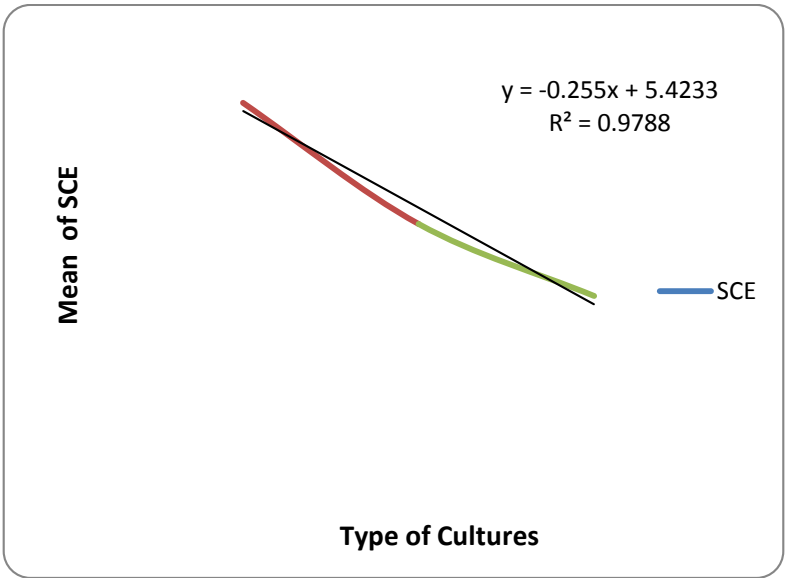

Fig.2. Total Mean values of SCE of Control and *Lawsonia inermis* extracts

Cell Proliferation Kinetics (CPK)

The preparation of slides for investigation of genotoxicity and used to determination of CPK. Twenty five number of metaphase spreads were identified per culture to measure the genotoxicity[18] of control and plant extracts using blood samples (BS) and shown in table 3 and figure 3.

Table 3

CPK of Control and *Lawsonia inermis* extracts using blood samples (BS)

Types of Culture	Control			500 µg/ml of plant extract			1000 µg/ml of plant extract		
	BS-1	BS-2	BS-3	BS-1	BS-2	BS-3	BS-1	BS-2	BS-3
M_i	5	7	7	5	9	9	7	7	8
M_{ii}	18	14	15	17	14	12	15	17	14
M_{iii}	4	5	6	3	5	7	4	2	4
Cell Proliferation Kinetics (CPK)	0.53	0.50	0.55	0.44	0.52	0.54	0.48	0.47	0.48
Mean (CPK)	0.52			0.50			0.48		

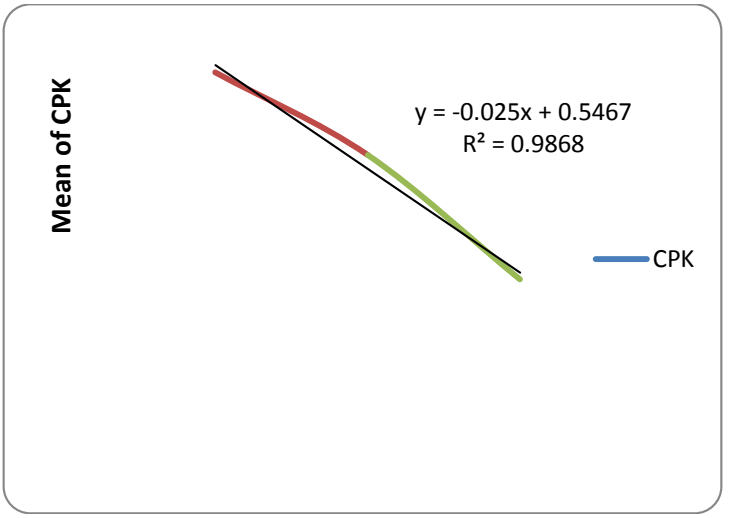

Fig.3. Total Mean values of CPK of Control and *Lawsonia inermis* extracts

The mean MI values of the cultures which revealed the results of two amounts of control and plant extracts and shown in table 1. Here the mean of control is 4.51 and 500µg/ml & 1000 µg/ml of *Lawsonia inermis* extracts of mean values are 4.33 and 4.18. The MI of *Lawsonia inermis* extracts were quite similar to the MI of the control. From the fig.1. Graphical representation of Type of culture and total mean of MI, equation was y = -0.165x + 4.67 and R^2 = 0.997, which indicates that MI of *Lawsonia inermis* extracts were not substantially dissimilar from the MI of control. The SCEs values of the cultures which revealed the results of two amounts of control and plant extracts and shown in table 2. Here the mean of control is 5.19 and 500µg/ml & 1000 µg/ml of *Lawsonia inermis* extracts of mean values are 4.87 and 4.68. The SCE of *Lawsonia inermis* extracts were quite similar to the SCE of the control.[19] From the fig.2. Graphical representation of Type of culture and total mean of SCE, equation was y = -0.255x + 5.423 and R^2 = 0.978, which indicates that SCE of *Lawsonia inermis* extracts were not substantially dissimilar from the SCE of control. In the CPK, the mean of control is 0.52 and 500µg/ml & 1000 µg/ml of *Lawsonia inermis* extracts of mean values are 0.50 and 0.48. The CPK of *Lawsonia inermis* extracts were quite similar to the CPK of the control. From the fig.3. Graphical representation of Type of culture and total mean of CPK, equation was y = -0.025x + 0.546 and R^2 = 0.986, which indicates that CPK of *Lawsonia inermis* extracts were not substantially dissimilar from the CPK of control. From the results, that the MI and frequency of SCES of ethanolic plant extracts are remarkably similar to that of control[20] which indicates that the *Lawsonia inermis* plant extracts have no substantial cytotoxicity and genotoxicity effects in cultured blood lymphocytes.

Conclusion

The blood sample culture was treated with control and various concentrations of extracts (500µg/ml and 1000µg/ml). The mean of MI, SCE and CPK for control were found to be 4.51, 5.19 and 0.52 respectively where as for 500µg/ml of *Lawsonia inermis* extracts are 4.33, 4.87 and 0.50 respectively and for 1000 µg/ml extracts are 4.18, 4.68 and 0.48 respectively. From the results, we can

conclude that the MI and frequency of SCES of ethanolic plant extracts are remarkably similar to that of control which indicates that the *Lawsonia inermis* plant extracts have no substantial cytotoxicity and genotoxicity effects in cultured blood lymphocytes.

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Conflict of interest

Authors do not have any financial or nonfinancial conflict of interest

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