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The Cytogenetic Effects of some agricultural waste extracts on Cultured Human Lymphocytes

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ABSTRACT: In this study, the cytogenetic effects of peel extracts of *citrus aurantium* L. (Bitter orange) and *citrus medica* L. (Lemon) were determined in cultured peripheral blood lymphocytes. Results showed that citrus aurantium L. (Bitter orange) and citrus medica L. (Lemon) Did not have a statistically significant effect on the micronucleus formation and mitotic index , counts indicated that Peel extracts had considerable anticlastogenic and antigenotoxic effects as observed in vitro in human lymphocytes. Thus, it could utilization of the waste products for therapeutic purpose and protected the normal cells from genotoxic or carcinogenic agents. As well as reduce pollution.

KEYWORDS: cytogenetic, lymphocytes, Agro waste; peels, chromosomal aberrations, Micronucleus, Mitotic Index.

1 INTRODUCTION

Citrus fruits are mainly used by juice processing industries while the peels are generally wasted. Since the juice yield of citrus is less half of the fruit weight, very large amounts of byproduct wastes, such as peels are formed every year(1). Peel waste are highly perishable and seasonal, is a problem to the processing industries and pollution monitoring agencies. So there will be take attention in bringing useful products from citrus waste materials. The citrus peels are rich in nutrients and contain many phytochemicals, these can be efficiently used as drugs or as food supplements. The peel of Citrus fruits is a rich source of flavonoid glycosides, coumarins , βand γ- sitosterol, glycosides and volatile oils (2). Many polymethoxylated flavones have several important bioactivities, which are very rare in other plants (3). In addition the fiber of citrus fruit also contains bioactive compounds, such as polyphenols, the most important being vitamin C (or ascorbic acid), and they certainly prevent and cure vitamin C deficiency-the cause of scurvy (4). Many studies have reported antioxidant and antibacterial effect of juice and edible parts of lemon and oranges of different varieties (5). As far as the peel is concerned, extracts from this part of the fruit were found to have a good total radical anti-oxidative potential (6). This study was aimed to minimize waste of fruit juice processing industries. The major waste part of lemon and oranges were peel, which were not used for any purpose. This research is for evaluating the component used as an antibacterial activity from waste peels of Citrus Such as (the sweet orange) Citrus aurantium (the bitter orange) .

2 MATERIALS AND METHODS

The plants used in this study were *Citrus aurantium* L.(Bitter Orange) and *Citrus medica* L. (Lemon). The peels were collected from the local fruit juice shops. After collection,and oven dried at 33°C for 7 days. The dried peels were powdered in an electric grinder and stored in plastic bags for the next step 100 gm sample of powder peels of oranges and lemon was extracted using 200 ml methanol (99.9%) in an electric blender for 30 min. This suspension was filtered .Then methanol was removed in a rotary evaporator to produce a dry powder. The final material was dissolved in methanol for obtaining concentrations of (10, 15 ,20) µg /ml

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CELL CULTURE ESTABLISHMENT AND SLIDE PREPARATION FOR THE CHROMOSOMAL ABERRATION ASSAY

Four fresh blood samples from the volunteers were collected, generally from the arm by venipuncture, and placed into a heparinised tube. Few drops of whole blood (0.5mL) are cultured in 5 mL of medium RPMI 1640 (pH 6.8 to 7.0), supplemented with 10% fetal calf serum, 10% antibiotic-antimycotic mixture and 1% phytohaemaglutinin of the final volume of cell culture . was added. peels extracts of 3 different doses (10,15 and 20 µg/ml) . And for mutagen cultures we were added Mitomycin-c 0.1 µg. After 24th hour , 0.1 ml (1 µg) on colcemid was added to the cultures just 90 minutes before harvesting. After addition of colcemid the cultures were incubated for 40 minutes and centrifuged at 1200 RPM for 10 minutes. To the pellet pre-warmed hypotonic solution (0.75M KCl) of 6ml was added. Incubated for 10 minutes and centrifugation was done at 1200 RPM for 10 minutes. and fixed in acetic acid : methanol (1:3 v/v). Chromosome preparations were stained with 3.3% Giemsa. The slides were analyzed at 1000 magnification using a light microscope. One hundred metaphases cells were screened per each individual. Cells with 46 chromosomes were scored for CA. The analysis of CA included chromatid and chromosome breaks, chromatid deletions, chromatid rings and dicentric chromosomes according to Api and San (7).

MITOTIC INDEX (MI) ASSAY

The slides were examined under high power (40 X) of compound light microscope and (1000) of divided and non-divided cells were counted and the mitotic index was calculated according to the following equation:

Mitotic index =no. of the dividing cells/ total no. of the cells (1000) ×100

IN VITRO MICRONUCLEUS ASSAY

Cells were exposed to different concentrations of the extract at 24 h culture. With the blood sample from each volunteer, four cultures were prepared, and treated with the extract 5, 10, 15 and 20 µg/mL in culture medium). The tested concentrations were established also in preliminary experiments. A negative control untreated culture and a positive control treated with 1.5 µg/mL from MMC

Cytochalasin B (6 µg/mL) was added at 44 h. After 72 h at 37ºC, cells were collected by centrifugation, rinsed and submitted to a mild hypotonic (1% sodium citrate) treatment and immediately fixed with methanol: acetic acid. Slides were prepared according to standard cytogenetic procedures and staining with 4% Giemsa. Slides were coded and scored by light microscopy at 400X or 1000X magnification as necessary. For each experiment, 2,000 binucleated lymphocytes with well preserved cytoplasm were scored. Micronuclei were identified according to the criteria of Fenech *et al.* (8).

3 RESULTS

MITOTIC INDEX

The mitotic index for the assessment of the cytotoxicity in the control and peel of *citrus aurantium* L. (Bitter orange) and *citrus medica* L. (Lemon) extracts of concentration 10 & 15 and 20 µg/ml using four blood samples for the accuracy.The Mean Mitotic Index (MI) values of the cultures which are exposed to three doses of plant extracts and control are obtained as shown in the Table 1. The mean value of MI of the control is 5.32 where as the peel extracts of *citrus aurantium* L. (Bitter orange) extracts have the mean value of MI of the 10 µg/ml is 4.89 and the mean value of MI of the 15 µg/ml is 4.96. and the mean value of MI of the 20 µg/ml is 5.15 , where as the peel extracts of *citrus medica* L. (Lemon) have the mean value of MI of the 10 µg/ml is 4.90 and the mean value of MI of the 15 µg/ml is 5.07. and the mean value of MI of the 20 µg/ml is 5.22. MI of both peel extracts were not much different from the MI of control. which indicates that MI of both peel extracts were not significantly different from the MI of control.

Table 1. Mitotic index (%) in human lymphocyte cultures exposed to extracts of peel extracts Bitter orang and Lemon

Test substance	Concentration µg /mL	MI%
Control	0	5.32
Positive con. (MMC)	0.25	2.28
Bitter orang	10	4.89
	15	4.96
	20	5.15
Lemon	10	4.90
	15	5.07
	20	5.22

MICRONUCLEUS

Showed Data of micronucleus analysis in binucleated lymphocytes are presented in Table 2. that the treatment of blood lymphoid cells with four concentrations of Lemon peel and bitter orange peel did not show significant variations In the frequency of micronuclei , when compared to negative control (p> 0.05).

Table 2 : Percentage change in lymphocyte micronucleus, according to the control and treatment with different concentrations of peel Bitter orang and peel Lemon

Test substance	Concentration µg /mL	Distribution of MN in BN				MN%
		0MN	1MN	2MN	3MN	
Control	0	998	2	0	0	0.2 a
Positive con. (MMC)	0.25	959	21	12	8	6.1 b
Bitter orang	10	993	7	0	0	0.7 a
	15	996	4	0	0	0.4 a
	20	994	6	0	0	0.6 a
Lemon	10	997	3	0	0	0.3
	15	998	2	0	0	0.2 a
	20	998	2	0	0	0.2 a

CHROMOSOMAL ABERRATIONS

Slides were prepared from each blood culture and 100 metaphase chromosome spreads were counted per culture was assessed to score for any chromosomal aberrations that may be induced by extract peels of concentration 10 & 15 and 20 µg/ml using four blood samples for accuracy.The Mean chromosomal aberrations values of the cultures which are exposed to three doses of peel extracts each of *citrus medica* L. (Lemon) , *citrus aurantium* L. (Bitter orange) and controlare obtaine as shown in Table 3. The mean value of (CA) of the control is 0.264 where as the *citrus aurantium* L. (Bitter orange) have the mean value of (CA) of the 10µg/ml is 0.301, and the mean value of (CA) of the 15 µg/ml is 0.312, and the mean value of (CA) of the 20 µg/ml is 0.26. but The mean value of (CA) of *citrus medica* L. (Lemon) of the 10µg/ml is 0.285, and the mean value of (CA) of the 15 µg/ml is 0.272, and the mean value of (CA) of the 20 µg/ml is 0.262 . (CA) of each peel extracts chromosomal aberrations were scored to measure the cytotoxic and genotoxic effects of the of citrus aurantium L. (Bitter orange) and citrus medica L. (Lemon) Test extract in cultures set up from the four different healthy donors. From the results, we can conclude that the and CA frequency of SCALES of peel extracts are almost similar to that of control which indicates

that the peel extracts of each citrus aurantium L. (Bitter orange) and citrus medica L. (Lemon) has no significant cytotoxicity and genotoxicity effects in cultured human peripheral blood lymphocytes.

Table 3:the effect of different concentrations of extract bitter orange peel and lemon peel on Chromosomal aberrations of human blood lymphocyte culture.

Test substances	Concentration (µg/mL)	Structural chromosomal aberration						Total
		Acentric	Dicenteric	Gap chromosome	Gap chromatid	chromatid Break	chromosome Break	%Aberrant
Control	0	0.0 a	0.0 a	0.020 a	0.244 a	0.0 a	0.0 a	0.264 a
Positive con. (MMC)	0.25	0.050 b	0.04 b	0.016 b	2.89 b	0.017 b	0.012 b	3.025 b
Bitter orang	10	0.0 a	0.0 a	0.080 a	0.290 a	0.0 a	0.0 a	0.301 a
	15	0.0 a	0.0 a	0.012 a	0.300 a	0.0 a	0.0 a	0.312 a
	20	0.0 a	0.0 a	0.018 a	0.250 a	0.0 a	0.0 a	0.268 a
Lemon	10	0.0 a	0.0 a	0.005 a	0.280 a	0.0 a	0.0 a	0.285 a
	15	0.0 a	0.0 a	0.072 a	0.200 a	0.0 a	0.0 a	0.272 a
	20	0.0 a	0.0 a	0.012 a	0.250 a	0.0 a	0.0 a	0.262 a

4 DISCUSSION

Fruits wastes and by-products, which are formed in great amounts during industrial processing, represent a serious problem, as they exert an influence on environment and need to be managed and / or utilized. On the other hand, they are very rich in bioactive components observe through our findings in this study that the citrus peel did not have a toxic or genetic effect in human lymphocytes in all concentrations used and possibly due to being a rich source of vitamin -C (Ascorbic acid). , And add to that the Citrus fruits peels is one of the important dietary sources of antioxidant phenolics so increased attention has been focused on the industrial wastes (9).

In summary, it could be concluded that the peel bitter orange and Lemon showed considerable antigenotoxic effects as observed in vitro in human lymphocytes. The peel bitter orange and peel (Lemon) could protect the normal cells from the genotoxic, or carcinogenic agents. This protection could be from the antioxidative roles of catechins, flavonoids, or other peels compounds (10). These findings supported the previous reports regarding the antioxidant effect of the peel bitter orange and peel Lemon. Further studies should be carried out to determine the effects of other components outside the main bioactive components isolated from the peels both bitter orange and Lemon in human lymphocytes cultures.

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