Genotoxicity of Jojoba (*Simmondsia chinensis*) Extracts Employing A Variety of Short-Term Genotoxic Bioassays

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ABSTRACT

Several short-term screening bioassays have been developed to detect mutagenic / carcinogenic potentiality of a substances especially that used as a food additive and or as a pharmaceuticals. At last decade jojoba extracts were widely used in cosmetics and in food additive as well. This work was planned to detect the possible genotoxic effect of jojoba (*Simmondsia chinensis*) extracts, defatted of jojoba seed meal (DJSM), defatted jojoba seed meal extracts (DJSE) and jojoba (JO) as well as the ability of DJSM and DJSE in reducing the body weight and the ability of JO as well as olive oil (OO) for reducing blood cholesterol. In order to achieve such a purpose, forty animals of albino mice (*Mus musculus* 2n=40) were used and divided into eight groups. The doses of treatments were, 3% DJSM; 0.2 ml and 0.1 ml DJSE; 0.2 ml JO; 0.1 ml JO; 0.2 ml OO and 0.05 ml JO + 0.05 ml OO in combination. Results showed that both 3% of DJSM and (0.2ml; 0.1ml) DJSE were effective in reducing body weight compared to untreated control. Jojoba seed oil significantly reduced both total cholesterol and low density lipoprotein for all tested doses of JO and OO. In addition the in vivo study demonstrated that no significant increase in micronucleated polychromatic erythrocytes (MNPCE) in the bone marrow of mice treated with DJSM, DJSE, JO or OO. The results obtained showed that oral administration of jojoba seed extracts at all doses did not caused a significant increase in either the percentage of micronucleated polychromatic erythrocytes in mice bone-marrow (MN test) or in the percentage of DNA aberration %. These findings might indicate that jojoba seed extracts does not induce damage for DNA in vivo, and so it has not any genotoxic effect. These results concluded that jojoba seed meal and its oil are safe for human usage at the level of the present work.

INTRODUCTION

Jojoba, *Simmondsia chinensis* 2n=52 (Link) Schneider, is a bushy shrub native to the arid areas, has emerged as a cash crop in India and other countries. It is a native of Northern Mexico and the Southwestern United States and grown commercially. Unspectacular in appearance, jojoba plants grow to 15 feet in height, with leathery, blue-green leaves about one inch in length and the plants can live for 200 years. Female jojobas produce oval fruits that open at maturity to reveal 1 – 3 brown, peanut-sized seeds. This plant is now cultivated in some countries: Argentina, Israel, USA and some Mediterranean and African lands. Empirically the Jojoba oil was used in folk remedies by the Indians in Mexico for cancer, kidney disorders, colds, dysuria, eyes, head, obesity, parturition, poison ivy, sore throat, warts, and wounds, according to Hartwell (1967–1971). However, it is not currently fed to animals because of the presence of several cyanide-containing glycosides, such as simmondsin, simmondsin 2'-ferulate, and several minor simmondsin derivatives (Booth *et.al.*, 1974; Elliger *et.al.*, 1973, 1974a, b; Van Boven *et.al.*, 1994a, b and c, 1995). These compounds cause reduced weight gains in ruminants (Manos *et.al.*, 1986) and death in mice (Weber *et.al.*, 1983) at levels of 1% in the feed. Simmondsin and simmondsin-containing jojoba meal induce food intake inhibition, emaciation and, occasionally mortality, and because of this, simmondsins have been considered toxic (Booth *et.al.*, 1974; Ngoupayou *et.al.*, 1982; Verbiscar *et.al.*, 1980, 1981). However, long-term administration of lower doses of simmondsin or defatted jojoba meal to growing rats, which induced a sustained food intake inhibition of about 20%, showed no toxic effects (Cokelaere *et.al.*, 1993a, b), although, real toxicity, especially at higher doses cannot yet be ruled out. Furthermore, it has been demonstrated that food intake inhibition in rats can be reversed by the cholecystokinin receptor antagonist, devazepide (Cokelaere *et.al.* 1995a, b), suggesting that the anorexia seen following simmondsin administration is due to stimulation of the cholecystokinin satiation system. Other studies have shown that the food intake reduction induced with lower doses of defatted jojoba meal is due to satiation (Cokelaere *et.al.*, 1995c).

The present work was planned to study.

1-The effect of defatted jojoba meal seeds on the body weight of mice (*Mus musculus*, 2n = 40.

2- The effect of jojoba oil seed on the cholesterol level of mice comparing to the olive oil.

3-The possible abnormalities of mice bone-marrow chromosome caused by jojoba extracts.

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MATERIALS AND METHODS

3-1: Effect of jojoba seed extract upon body weight of mice.

3-1-A: Solid- liquid extraction process of jojoba seeds extracts:

Jojoba seeds (150g) used in this work was brought from Egyptian Jojoba farm in Wady El-NatronEl-Behera governorate. The oil content of the meal was determined by complete extraction using Soxhlet extractor for 16 hour and it was found to be 50/100 (v/w). Solvents used for oil extraction were commercial hexane. Defatted of jojoba meal (25g) was ground in a coffee mill to obtain a finely divided material suitable for extraction studies. Aqueous solutions of 80-100% ethanol were prepared from analytical grade absolute ethanol and deionized water. Samples of defatted jojoba meal were weighed into glass screw-cap vials and the appropriate volume of solvent was delivered by pipette to obtain a 1: I0, meal to solvent ratio, cover; blend and let for 30 minute then pour extract into fluted filter paper assembly. The supernatant was evaporated by a water bath then the pellet was dissolved in 5 ml of 95% ethanol and diluted with 30 ml of tap water to final concentration.

3-1-B: Treatments:

Twenty animals of mice were primarily weighted and then were divided into four groups each five replicates, the first and second group were ingested orally with 0.2ml and 0.1ml solution of defatted jojoba seeds extracts /mouse respectively one time daily, while the third group was feed by a diet supplemented with 3% of defatted jojoba seeds. The animals of the negative control group had received the proper volume of deionized water (0.2ml). Thirteen days after, animal were weighted again. The animals were killed by decapitation 24 hr after the last dose, three hours prior to killing the animals were injected with 0.4mg/kg b.w.t. of colchicine.

3-2: effect of jojoba seed oil and olive oil upon the level of mice blood cholesterol.

Treatments:

Twenty four animals were grouped into five treatments four animals were used for each treatment. Each animal had orally received the proper dose one time daily for 30 days. Where the first and second group treated with 0.2ml and 0.1ml of jojoba oil (JO), respectively the third group were treated with 0.2ml of olive oil (OO)/ mouse, the fourth group were received 0.05ml of JO + 0.05 ml OO /mice. Animals of the negative control group received equivalent amounts of demonized water. The animals were killed by decapitation 24 hr after the last dose. Three hours prior to killing, the animals were injected with 0.4 mg/kg of colchicine.

Plasma Cholesterol and Triacylglycerol Levels:

Mice were fasted for 8 hours before collection of venous blood from the retro-orbital sinus into a heparinised capillary tube. Fasting plasma samples were obtained for total cholesterol and triacylglycerol determinations at baseline, at the end of the study (4 weeks). Plasma was isolated by centrifugation at 3000 xg for 10 minutes at 4°C and stored at -20°C. Enzymatic measurements of total cholesterol and triacylg-lycerol levels were performed using standard methods at the clinical chemistry department of Mabaret Alasafra Hospital, Alexandria.

3-3 A: Analysis of chromosomal behavior in mice bone-marrow.

Mice originally colonized from the NAMRU-3 and bred at the University of Alexandria Research Center (UNARC) were used in this part of the present study. This healthy random bred strain maximizes genetic heterogeneity and at the same time assures access to a common source. Each animal had orally received the proper dose one time daily for 30 days. In this work twenty eight animals were divided in to seven groups, four mice were used for each treatment. The first group were received 0.2ml of defatted Jojoba meal extract, where second group were fed on fattening feed supplemented with 3% of defatted Jojoba seed meal; the third and fourth group received orally 0.2 and 0.1 ml of Jojoba seed oil respectively, the fifth and sixth group were ingested with 0.2 and 0.5 ml of Jojoba oil+ 0.5 ml of olive oil respectively, the last group (un treated) were left as a control. The animals were killed by decapitation 24 hr after the last dose. For each treatment, four animals were used. Animals of the control group received equivalent amounts of demonized water. Three hours prior to killing, the animals were injected with 0.6 mg/kg of colchicine (Seehy & Osman 1989).

Extraction of bone-marrow:

After killing, the adhering soft tissue and epiphyses of both tibiae were removed. The marrow was aspirated from the bone, transferred to phosphate buffered saline, centrifuged at 1000 rpm for 5 minutes and the pellet resuspended in 0.075 M KCl. Centrifugation was repeated and the pellet was resuspended in fixative (methanol: acetic acid, 3:1). The fixative was changed after 2 hours and the cell suspension was left overnight at 4 0C.

Slide prepartion and staining:

Cells in fixative were dropped on very clean glass slides and air-dried. Spreads were stained with 10 % Giemsa at PH 6.8 for 5 min.

Screening of slides:

Slides were coded and scored for chromosomal aberrations e.g., gaps and deletion, fragment, break, stickiness and polyploidy. A mitotic index based on at least 1000 counted cells was recorded. For chromosomal abnormalities at least 200 scorable metaphase cells per dose were recorded. Comparison with negative control was also statistically tested when needed.

Micronucleus test in mice:

Male and female of strain Swiss albino from randomly bread colony were used in the micronucleus assay. Four animals were used for each dose. Each animal had received the proper dose jojoba seed extract. Control group had received the proper volume of deionized water.

Preparation of the smears:

Bone-marrow smears were made according to Schmid (1975). The tubes were centrifuged at 1000 rpm for 5 min., the supernatant was removed and a small drop of the viscous pellet was transferred on the end of a slide and spread by a cover glass held at an angle of about 45degrees. The preparation was then air-dried.

Staining:

The preparations were stained in ordinary vertical staining jar according to method described by Gallapuidi and Kamara (1979). The slides were fixed in absolute methanol for 5 min., rinsed twice in deionized distilled water, stained for 10 min. in Giemsa (1:6 Gurr's R-bb Giemsa in deionized water), rinsed again thoroughly in deionized distilled water, air-dried, cleaned in xylene for 3 min and mounted.

Screening of slides:

A thousand polychromatic erythrocytes per animal were scored using a special hand counter. The frequency of micronucleated cells was expressed as percent of micronucleated cells based on the total polychromatic erythrocytes percent.

In this study, only polychromatic erythrocytes were scored according to Brusick (1986). Micronuclei were identified as dark-blue staining bodies in the cytoplasm of polychromatic erythrocytes. The data obtained from this study were analyzed according to Hart and Pederson (1983).

RESULTS AND DISCUSSIONS

Jojoba effects on food intake and body weight.

Data in Table (1) show that the mean body weight of animals was reduced (11.8%) after feeding diet supplemented with 3% (wt/wt) of DJSM while the percentages of body weight loss were 8.7-11.2% for the animals ingested orally with 0.1 and 0.2 ml of DJSE, respectively at the end of experiment. On the other hand, the animals fed the control diet gained weight progressively throughout the experiment (9.14%).

Table 1. Mice body weight treated with defatted jojoba seed meal (DJSM) and defatted jojoba seed extract (DJSE)

Treatment & dose	Body weight (g)		Change %
	Before treatment	After treatment	
Control	20.8	22.7	+ 9.14
DJSM; 3%	21.2	18.7	- 11.8
DJSE; 0.2 ml	20.5	18.2	- 11.2
DJSE; 0.1 ml	20.6	18.8	- 8.7

DJSM = defatted jojoba seed meal; DJSE = defatted jojoba seed extract. Means are average of 4 mice.

Effects of jojoba seed oil and olive oil on mice cholesterol level.

Tables (2) illustrate the level of total cholesterol (CH); low density lipoprotein (LDL); high density lipoprotein (HDL) and triglycerides (TG). Its clear that jojoba seed oil (JO) was found to be very effective in inducing significant decreases in both LDL and TG as well as in total cholesterol. On the other hand, it was found that high density lipoprotein was increased for the group treated with JO. Despite of decreased of LDL, TG was increased where olive oil had used compared to the control group. The highest values of CH and TG were detected for the control group and the group treated with olive oil (206.7, 177 and 196.33, 224 mg/dm), respectively. The lowest level in LDL were recorded for groups treated with 0.1 ml 0.2 ml of JO and group treated with 0.05 ml JO + 0.05 ml OO (-82.86, -30.37 and 7.66), respectively. That's values were related to the significant increase of HDL, concluded that JO are effective in reducing blood cholesterol.

1- Cell proliferation:

Data obtained are given in (Table 4). Metaphase index were 3.1% and 3.4% for the untreated mice (negative control) and the group treated with 0.2 ml DJSE/mouse, respectively. While this percentage was decreased to be 2.03 and 2.4% for the group treated with 0.2 ml and 0.1 ml of JO/mouse, respectively. The highest increase in metaphase indeces was recorded for the group treated with 3% DJSM.

Micronucleus test:

Data in Table (4) showed that there were no significant differences in the percentage of micronucleated polychromatic erythrocytes for the groups treated with DJSM; DJSE or JO compared to the untreated control.

Table 2. Total cholesterol (CH); triglycerides (TG); high density lipoprotein (HDL); and low density lipoprotein (LDL) of mice blood treated with jojoba oil and olive oil

Treatment	Blood analysis (mg/dm)			
	CH	TG	HDL	LDL
Control	206.70	177	103.30	67.93
0.2 ml JO	124	120.50	130.27	-30.37
0.1 ml JO	112	107.66	173.33	-82.86
0.2 ml OO	196.33	224	102.66	48.80
0.05 ml JO + 0.05 ml OO	128	130	94.33	7.66

Data represent an average of 3 replicates.

Table 3. Metaphase indices in mice bone-marrow after treatment with jojoba seed extracts

Treatment	Total counted cells	Metaphase cells	Metaphase index	Change%
Control	2973	92	3.1 %	-
3% DJSM	2287	143	6.3 %	+103.2
0.2 ml DJSE	4394	148	3.4 %	+9.7
0.2 ml JO	6464	131	2.03 %	-34.5
0.1 ml JO	3331	80	2.4 %	-22.6

DJSM: defatted jojoba seed meal; DJSE: defatted jojoba seed extracts and JO: jojoba oil. Means are average of 2000 cells.

Table 4. Micro-nucleated polychromatic erythrocytes (MNPE) in mice bone-marrow cells after treatment with jojoba seed extracts, DJSM, DJSE or JO

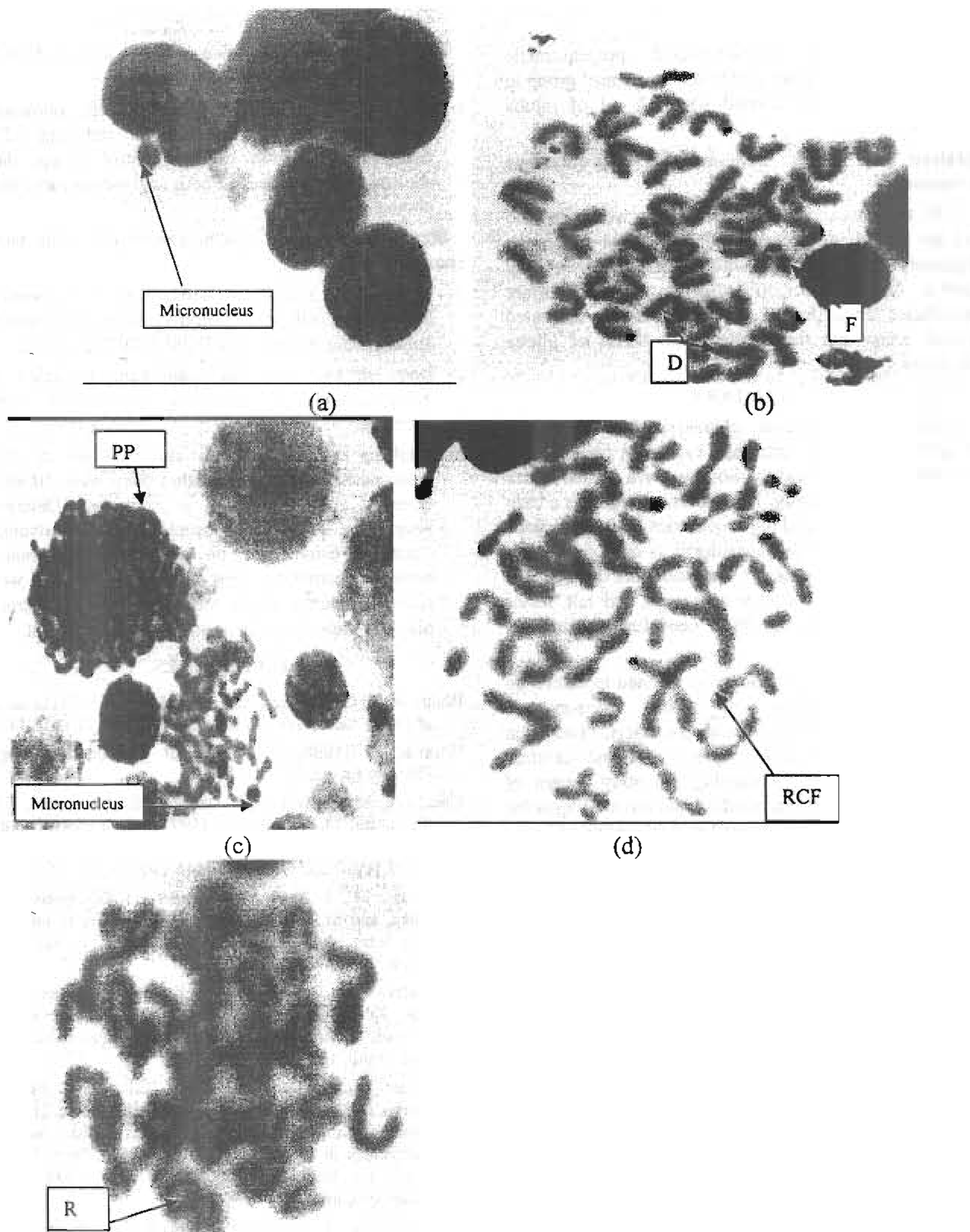
Treatment	No. of MNPE	MNPE %
Control	7	0.35
DJSM; 3%	9	0.45
DJSE; 0.2 ml/mouse	19	0.95
DJSE; 0.1 ml/ mouse	12	0.60
JO; 0.2 ml/mouse	21	1.05
JO; 0.1 ml/mouse	11	0.55
JO;0.05 ml+ OO;0.05ml	9	0.45
OO; 0.2 ml/mouse	10	0.50

DJSM=defatted jojoba seed meal; DJSE= defatted jojoba seed extract; JO= jojoba oil; OO= olive oil. MNPE % = No of MNPE/total No. of counted cells (2000 cells).

Table 5. Percentage of mice chromosomal aberration after treatment with jojoba extracts (200 counted cells)

Treatment & dose	No. of aberration / 200 cells					Total	Aberration %
	Stickiness	Deletion	Fragment	RCF	Ring chromosome		
Control	3	0	0	1	1	5	2.5
0.2 ml JO	14	1	3	2	2	22	11
0.1 ml JO	6	1	1	3	2	13	6.5
0.2ml OO	3	2	0	2	0	7	3.5
0.05 ml JO+0.05 ml OO	4	2	2	1	0	9	4.5
3% DJSM	3	1	0	1	2	7	3.5
0.2 ml DJSE	7	2	1	3	0	13	6.5
0.1 ml DJSE	4	3	0	2	1	10	5

DJSM=defatted jojoba seed meal; DJSE= defatted jojoba seed extract; JO= jojoba oil; OO= olive oil. RCR= Robertsonian Centric Fusion.



Figue 3. Photomicrographs showing a- micronucleus; b- chromosome deletion (D) and fragment (F); c- polyploidy (PP) and micronucleus; d- Robertsonian Centric Fusion (RCF) and e- Ring chromossome (R)

Percentages of micronucleated polychromatic erythrocytes ranged from 0.35% for the control group to 1.05% for the group treated with 0.2 ml of jojoba oil/mouse.

Analysis of mice bone-marrow chromosome behavior:

All types of chromosome aberration were observed and are shown in Figures (4-8) They were stickiness; d-fragment; deletion; ring chromosome; Robertsonian Centric fusion; chromatid deletion. Total aberrant metaphases ranged from 2.5% to 11% for the untreated control group and that treated with 0.2ml of jojoba oil/mouse

SUMMARY

Jojoba (*Simmondsia chinensis*, 2n=52) (Link) Schneider is a bushy shrub and evergreen plant, native to Northern Mexico and the Southwestern United States and grown commercially. It has been emerged as a cash crop in India, Israel and other countries. In Egypt jojoba is anew exotic plant and considered as one of the most practical solutions for desert plantation and this because its ability for tolerance to heat, drought and salt, lesser possibilities for infection, lesser need for fertilizers, as well as be consider

The present investigation was planned to detect the possible abnormalities of mice bone-marrow chromosome caused by jojoba seed extracts. In addition the capability of defatted jojoba seed and defatted jojoba seed extracts in reducing the body weight of mice. The effect of jojoba seed oil and olive oil upon the level of total cholesterol as well as triglyceride of blood mice was tested. In order to achieve such a purpose, mouse *(Mus musculus)* 2n=40 was chosen as a sensitive monitor for the possible genotoxic effect of jojoba extracts. The following short term genotoxic bioassays were selected and used.

1- Estimation of cell proliferation activity.
2- Analysis of micronucleated polychromatic erythrocytes in mice.
3- Analysis of chromosomal abnormalities in mice bone-marrow cells.

The obtained results could be summarized as follows:

a- Defatted of jojoba seed meal (DGSM) supplemented to feeding diet 3% (w/w) as well as defatted jojoba seed extracts (DJSE) at the dose level of 0.2 ml and 0.1 ml / mouse were proven to be significantly reduced body weight of mice compared to the negative control group.

b- Jojoba seed oil was effective in reducing both total cholesterol; LDL and triglycerides with high increases of HDL in mice blood at a level dose of 0.1 ml and 0.2 ml of JO / mouse.

c-Although the olive oil at a level dose of 0.2 ml/mouse was proven to be effective in reducing LDL compared with that of the control group, data showed high increases in both triglycerides and total cholesterol as well.

Short-term genotoxic bioassays employed in this work showed that:

1- There were significant differences in metaphase indices and cell proliferation in mice bone marrow animals after treated with DGSM; DJSE and JO
2- Data showed that no significant increases in micronucleated polychromatic erythrocytes were observed
3- Analysis of chromosomal abnormalities in mice bone-marrow cells showed that there were different types of aberrations such as Stickiness; Deletion; Fragments; Ring Chromosomes and Robertsonian Centric Fusion but no significant differences between treatment groups and negative control were recorded such a result means that jojoba extracts plays an important role as anti-clastogenic agent.

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الملخص العربي

السمية الوراثية لمستخلصات الجوجوبا بتوظيف مجموعة إختبارات سمية وراثية

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نباتات الجوجوبا(*Simmondsia chinensis*) تحتوى العدد الكروموسومى ٥٢ وهى شجيرة كثيفة مستديمة الخضرة وقد تم أكتشافها إبتدائا فى شمال المكسيك والجنوب الغربى للولايات المتحدة الأمريكيه. وقد تم زراعتها كمحصول إقتصادى فى كل من الهند وإسرائيل وبعض دول العالم وفى مصر تعتبر الجوجوبا حلا نموذجيا لزراعة الصحراء المصرية وذلك بسبب قدرتها على تحمل ظروف الجفاف وندرة المياة وإرتفاع الحرارة فى بيئة الصحراء المصرية وكذلك بسبب ضعف إمكانية إصابتها بالأمراض وقلة إحتياجاتها من التسميد.

وقد تم تصميم هذا البحث بغرض

١- تحديد إمكانية وجود خلل فى السلوك الكروموسومى لكروموسومات خلايا نخاع العظام فى فئران المعمل البيضاء.

٢- قدرة كل من مسحوق بذور الجوجوبا منزوعة الزيت (DJSM) وكذلك مستخلص هذا المسحوق (DJSE) على خفض وزن فئران التجارب.

٣- تأثير كل من زيت الجوجوبا(JO) وزيت الزيتون(OO) على مستوى الكوليسترول الكلى وكذلك مستوى التراي جليسريدات فى دماء فئران المعمل.

ولتحقيق هذا الغرض تم إستخدام فأر المعمل من النوع *Mus musculus* والذى يحتوى العدد الكروموسومى ٤٠ ككاشف حساس لتحديد السمية الوراثية لمستخلصات نبات الجوجوبا.

حيث تم إستخدام مجموعة إختبارات سمية وراثيه قصيرة المدى وهى كالتالى:-

١- تقدير معامل الأنقسام (نسبة الخلايا المنقسمة)

٢- تحليل سلوك النواة الصغيرة

٣- تحليل الشذوذات الكروموسومية فى خلايا نخاع عظام الفئران

وقد أظهرت النتائج ما يلى:

١- قدرة كل من مسحوق بذور الجوجوبا الخالى من الزيت(DJSM) والمضاف لغذاء الفئران بمعدل ٣% وزن/وزن وكذلك مستخلص مسحوق بذور الجوجوبا منزوعه الدهون(DJSE) عند إستخدامها بمعدل٠,٢و٠,١ ملى/ فأر على خفض وزن الفئران مقارنة بالمجموعة الضابطة الغير معاملة.

٢- فاعلية زيت الجوجوبا(JO) على خفض مستوى كل من الكوليسترول الكلى وLDL وأيضا التريجليسريدات مع زيادة فى مستوى HDL فى دماء الفئران عند إستخدام زيت الجوجوبا بمعدل ٠,٢و٠,١ ملى/ فأر.

٣- على الرغم من أن زيت الزيتون عند إستخدامة بمعدل ٠,٢ ملى/ فار كان فعال فى خفض نسبة LDL مقارنة بالمجموعة الضابطة. إلا أن النتائج اظهر زيادة عالية فى كل من الكوليسترول الكلى والتريجليسريدات.

-وبالنسبه لإختبارات السمية الوراثية قصيرة المدى فقد أظهرت النتائج ما يلى

١- وجود إختلافات معنوية فى معدل إنقسام الخلايا نتيجة المعاملة بكل من DJSM,DJSE,JO مقارنة بالمجموعة المقارنة.

٢- عدم وجود إختلافات معنوية بين المعاملات والمجموعة الكنترول بالنسبة لإختبار النواة الصغيرة.

٣- بالنسبة لتحليل السلوك الكروموسومى لكروموسومات خلايا نخاع عظام الفئران فقد تم تحديد مجموعة مختلفة من الشذوذات فى حالة المعاملة بمستخلصات بذورالجوجوبا ومنها اللزوجة والنقص والشظايا الكروموسومية والكروموسومات الحلقية وكذلك (RCF) إلا أن نسبة تكرارهذة الشذوذات فى المجاميع المعاملة لم يكن بينها وبين المجموعة المقارنة إختلافات معنوية.