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CYTOGENETIC ACTIVITY AND ANTIOXIDANT ROLE OF FLAXSEEDS (*LINUM USITATISSIMUM* L.) EXTRACT IN MALE ALBINO RATS TREATED WITH CYPROTERONE ACETATE

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ABSTRACT

Objectives Investigation the cytogenetic activity and antioxidant role of methanolic extract of flaxseeds in male albino rats treated with cyproterone acetate.

Methods The first part contains a phytochemical analysis of flaxseeds 20% methanolic extract include: Thin layer chromatography (TLC), determination of active compounds, and antioxidant activity of flaxseeds extract. The second part was experimental groups include 35 rats divided randomly into 7 groups: first and second groups treated with distilled water (D.W) and corn oil respectively as control groups, cyproterone acetate (CPA) (10 mg/kg/day) group, flaxseeds extract (250 and 500 mg/kg/day) respectively, and groups treated both CPA with flaxseeds extract with the same doses above respectively. After 50 days, the cytogenetic study including mitotic index (MI) and chromosomal aberrations (CA) of bone marrow cells in addition to the levels of DNA fragmentation in both white blood cells (WBC) and testes tissues was assessed.

Results Presence of some phytochemical and antioxidant compounds in 20% methanolic extract of flaxseeds. CPA group had a significant increase ($P \leq 0.05$) in MI and CA compared to both D.W and corn oil control groups and has a high level of DNA fragmentation in both WBC and testes tissue. In contrast, flaxseeds extract alone or with CPA did not induce a significant difference in MI and CA but it considered as significant decreasing ($P \leq 0.05$) when compared with CPA group. Furthermore, both groups treated with flaxseeds 250 and 500 mg/kg/day alone did not record any fragmentation of DNA of WBC but caused fragmentation of DNA of testes tissue. While a decreased level of DNA fragmentation of WBC and testes tissue was noted in CPA with flaxseeds treated groups when compared with the CPA group.

Conclusions The methanolic extract of flaxseeds had a cytogenetic activity to suppresses mitogenic and genotoxic effects of CPA via its antioxidant properties.

Keywords cyproterone acetate, flaxseeds, chromosomal aberrations, mitotic index, antioxidant activity.

INTRODUCTION

Cyproterone acetate (CPA) has trade name like ae Androcur, Cyprostat, and Siterone, are synthetic sex steroidal antiandrogen, progestin, and antigonadotropin (Neumann and Topert, 1986; Kasper, 2001). The chem-ical formula of CPA ($C_{24}H_{29}CLO_4$). It is metabolized by two processes: hydroxylations and glucuronide conjugation and the final product of its metabolism is 15-β-hydroxycyproterone acetate (Hellerstedt and Pienta, 2002). Actually, CPA act as a competitive inhibitor of dihydrotestosterone (DHT) and testosterone at sites of the receptor of androgen. Antiandrogen can operate directly on fertility that inhibits action of testosterone and dihydrotestosterone (DHT) in the tissue by negative feedback mechanism and this lead to closure of the androgen receptor (AR) and it can remain high in the blood and this may lead to inhibition of luteinizing hormone (LH) and then lost of the testosterone (Sciarra et al., 1990, Falsetti et al., 2001). Antiandrogen CPA had been confirmed in therapy for precocious puberty, dermatological conditions related to androgen like seborrhea, acne, androgenic alopecia, and hirsutism, and for prostate cancer (Maggi, 2011). Antiandrogens have a molecular mechanism of action begins by antiandrogens link to the AR (which is transcription factor and reactive whereas androgens were found) at ligand binding pocket and thereby prevent its activation (Helsen et al., 2012). As a result of androgen binding, it initiates expressing of genes which include response elements, that is identified by the AR (Denayer et al., 2010). Furthermore, CPA can inhibit hormone receptor complex translocation into the nucleus of cell (Sciarra et al., 1990; Falsetti et al., 2001). Progestational activity of CPA is well known and it has been utilized by oneself or union with estradiol valerate or ethinyl estradiol for therapy of females have hyperandrogenism such as acne or hirsutism (Sciarra et al., 1990; Shrivastava and Agrawal, 2016). Several side effects were linked to CPA itself as weight gain and headache (Czerny et al., 2002), elevated concentration of prolactin, adrenal failure or hyperplasia (0.5% of cases) mainly depicted in juveniles take CPA (Laron and Kauli, 2000). Also, reduced tolerance of glucose, pituitary and kidney dysfunction, and anaemia (Hill *et al*., 2003). Animal treated with CPA (10 and 5 mg/kg) showed significant increases in total cholesterol, triglycerides, low-density lipoprote-ins, IgG and neutrophils and a significant decrease in lympho-

cytes was reported when given CPA (10 mg/kg) (Al-Sa'adi, 2010).

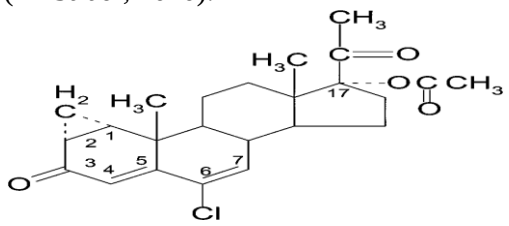

Figure 1: **Chemical structure of cyproterone acetate (Kasper, 2001).**

In addition, side effects of CPA in males includeing general feminization, breast enlargement and pain, the outflow of milk, sexual malfunction as loss of lib-ido, impaired spermatogenesis, erectile malfunct- ion, and reversible infertility (Iversen *et al*., 2001; Wakelin, 2002). Furthermore, CPA had an eleva-ted relationship with depression in males and females (Wakelin, 2002). Maggi (2011) had been mentioned that about 20–30 % of females treated with CPA (dosage range 25–100 mg) for hirsut-ism has shown symptoms of depression.

However, severe drawbacks like hepatotoxicity, cardiovascular toxicity (4-40% for CPA), and hypertension had limited their clinical utilization (Reilly *et al*., 2000; Savidou *et al*., 2006; Mottet *et al*., 2015).

The genotoxicity of CPA includes the chromosomes aberrations (CA) which observed as chromosome and chromatid breaks with chromatid exchanges have been seen at 20 and 30 µM of CPA (Siddique and Afzal, 2004). Martelli *et al.* (1995) showed DNA adducts formation can detect in human hepatocytes treated with CPA of four female and two male donors. Also, CPA induced a genotoxic effect on human lymphocytes by studying CA, sister chromatid exchanges (SCE) and both replication and mitotic index (MI) as indicators when using of CPA (30 µM) and propose that CPA had genotoxicity on lymphocytes of human *in vitro* (Siddique *et al*., 2007). Thi genotoxicity of CPA was detected as a potential function of reactive oxygen species (ROS) (Siddique and Afzal, 2005).

Flaxseeds (also has the name linseeds) *(Linum usitatissimum* L.) belongs to *Linaceae* family (Zhang *et al.,* 2008). It had golden and brown varieties and the flaxseeds shape is oval or flat up to (4–6) mm in size with a sharp tip (Daun *et al*., 2003; Bernacchia *et al*., 2014). Flaxseeds were one of the world oldest cultivated plants and were one of the abundance plants of ω-3 fatty acids known to human which use for medicine, textiles, and food. Flaxseeds contain a good quantity of dietary fiber, α-linolenic acid, omega-3 fatty acid, protein, lignan particularly Secoiso-lariciresinol diglucoside (SDG) (Collins *et al*., 2003; Gutte *et al*., 2015). Analysis of flaxseeds averaged 20–25 % of protein, 30–40% of oil, 20-28% of total dietary fiber, 3–4% of ash and 4–8% of moisture. The oil includes vitamins A, B, D and E, amino acids and minerals. Flaxseeds had much potential health benefits and therapeutic uses as a whole flaxseeds, lignan precursors, flaxseeds oil, and it has numerous potential advantage in the treatment or prevention of different diseases (Amin and Thakur, 2014) like inhibition of cardiovascular disease, diabetes, atherosclerosis, arthritis, cancer, osteoporosis, neurological and autoimmune disorders. (Zhang *et al.*, 2008; Jhala and Hall, 2010; Goyal *et al*., 2014; Gutte *et al*., 2015). Furthermore, flaxseeds had anticancer influences in colon, prostate, and breast cancers (Jhala and Hall, 2010). Furthermore, they can offer antiviral, anti-inflammatory effect, bactericidal activity, and had an impact on bone health (Zhang *et al.,* 2008).

Materials and Methods

Plant Collection and Identification: Flaxseeds used in this study were purchased from local herbal markets. The plant was identified by Plant Herbarium/College of Science/Department of Biology/ /University of Babylon.

Figure 2. **Flaxseeds used in the study.**

Preparation of Plant Extract: The plant extract was prepared according to Sato *et al*. (1990).

Preparation of Cyproterone Acetate: Androcur was a trade name of CPA used in this study which obtained from local pharmacies and supplied from the company of a subsidiary of Filiale de Schering AG/Germany, as 10 mg concentration for each tablet. Crushed tablets soluted in absolute ethyl alcohol and left exposed to the air until drought then corn oil was added and required concentrations to conduct experiments were calculated depending on the dose given to human (Abd-Al-Ameer, 2008).

Phytochemical analysis: The preliminary qualitative phytochemical analysis was performed by recognizing the secondary metabolites found in this extract. The procedure already described by

Joshi *et al*. (2013) was applied for alkaloids while the presence of glycosides, resins, and determination of pH of the extract was judged as Shihata (1951). Terpenes and steroids were detected by the procedure described by Al-Maisary (1999). Saponins were calculated by two different methods described by Stahle (1969) and Shihata (1951). In addition, coumarins, essential oils, flavones, phenolic compounds, and tannins were detected according to the following references respectively (Geisman, 1962; Indian Herbal Pharmacopeias, 1998, Jaffer *et al*., 1983, Harborne, 1984, Al-Shami, 1982).

Thin Layer Chromatography (TLC): TLC technique was applied by using mobile phase systems, as following: Ethyl acetate: benzene (1:6 v/v), Ethyl acetate: ethanol (1:19 v/v), and chloroform: ethyl acetate (80:20 v/v). It was visualized by both visible and UV light (Vekiari *et al*., 1993).

Antioxidant Activity Test (β-Carotene Spray): The β-carotene spray was carried out according to Pratt and Miller (1984).

DPPH Radical Scavenging Activity Test: To estimate antioxidant activity, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was utilized. After the scavenging of DPPH, the reduction in absorbance at 515 nm which take place in consequence of the inhibition by the antioxidant was observed. DPPH is greatly utilized to ensure the capability of compounds to perform as free radical scavengers or donors of hydrogen and to estimate the anti-oxidant activity of foods (Taha, 2010).

Study Design: In this study, thirty-five adult white male rats aged 2-3 months were used. The animals were supplied with both food and water *ad libitum*. After adaptation, the rats were randomly separated into 7 groups and gave a treatment orally for 50 days as follow:

- First and second control groups treated with one ml of distilled water and corn oil respectively.
-The third group treated with cyproterone acetate (10 mg/kg)(Al-Sa'adi, 2010). -
Fourth and fifth groups treated with crude flaxseeds extract (250 and 500 mg/kg) respectively (Ahmad, *et al*., 2012, Nagar and Vidyapeeth, 2014).
Sixth and seventh groups treated with CPA 10 mg with crude flaxseeds extract 250 and 500 mg/kg respectively.

All rats have been sacrificed 24 hours after the last dose and drugged by diethyl ether and then has been opened by a scalpel and sharp scissors. The blood was taken directly by heart puncture and was put into EDTA tube and part of testis was frozen for DNA extraction. Also, bone marrow was taken for cytogenetic study.

Cytogenetic Study: The cytogenetic study was performed according to a method of (Sharma and Sharma, 1980). At least 1000 metaphase cells per animal were scored to investigate chromosomal aberrations and mitotic index. Mitotic index was determined from each animal according to the following formula:

$$\text{Mitotic index (MI)} = \frac{\text{No.of divided cells}}{\text{Total No.of scorded cells}} \times 100$$

DNA Extraction from WBC, Testes Tissues, and Agarose Gel Electrophoresis: Blood samples and testes tissues were obtained from both treated and control groups and genomic DNA from WBC and testes tissues extracted by using DNA extraction kit Favorgen and Promega kits respectively and according to manufacturer's instructions. Electrophor-sis was undertaken under 75 (Robinson and Lafleche, 2000).

Statistical Analysis: Analysis of data was performed by utilizing Statistical Package for Social Science (SPSS) system/ version 22. Results described as mean±S.E. The Analysis of Variance (ANOVA) and Duncan was utilized to make a comparison between means and percentage.

RESULTS

Table 1 shows that some phytochemical compounds were found in flaxseeds extract and its pH which was 7.24. The figures 1- 3 and Tables 2-4 explain the numbers, properties, and R_f values of each band for the extract.

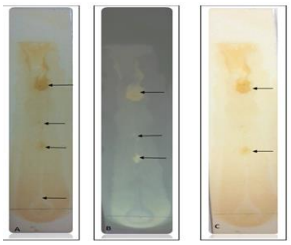

Figure 3: TLC of 20%methanolic extract of flaxseeds using developing solvent system ethyl acetate : benzene (1:6 v/v). A: visiblelight. B: UV light. C: antioxidant bands

Table 1: Detection of phytochemical compounds in 20% methanolic extract of flaxseeds. extract.

Phytochemical Compound	Flaxseeds extract
Alkaloids	+
Coumarins	+
Essential oils	+
Flavones	+
Glycosides	_
Phenolic compounds	+
Resins	+
Saponins	+
	+
Tanins	_
Terpens	+
Steroids	+
PH	7.24

Table 2**:** TLC of 20% methanolic extract of flaxseeds using developing solvent system ethyl acetate:benzene (1:6 v/v).

Detection Method	Bands properties		Number of bands
	Color	**R_f value**	
Visible light	white	0.16	4
	yellow	0.36*	
	yellow	0.50	
	brown	0.72 *	
UV - light	white	0.36*	3
	white	0.50	
	pale yellow	0.72*	
β-carotene	Yellow	0.36*	2
		0.72*	

Table 3: TLC of 20%methanolic extract of flaxseeds using developing solvent system ethyl acetate: ethanol (1:19 v/v).

Detection Method	**Bands properties**		**Number of bands**
	Color	**R_f value**	
Visible light	White	0.07	2
	Yellow	0.74*	
UV - light	Blue	0.13	3
	White	0.57	
	Brown	0.74*	
β-carotene	Yellow	0.74*	1

Table 4: TLC of 20%methanolic extract of flaxseeds using developing solvent system chloroform: ethyl acetate (80:20 v/v).

Detection Method	Bands properties		Number of bands
	Color	**R_f value**	
Visible light	Yellow	0.28*	3
	White	0.54	
	Brown	0.84	
UV – light	Pale brown	0.08	5
	Green	0.15	
	White	0.28*	
	Purpule	0.84	
	Gray	0.93	
β-carotene	Yellow	0.28*	1

Antioxidant Activity assays: β-Carotene assay: After a spray of the silica gel with β-carotene, the results which showed the antioxidant activity of extract were represented by bands which presserved the yellowish color. A number of antioxidant bands were 2, 1 and 1 in developing solvent systems ethyl acetate: benzene (1:6 v/v), ethyl acetate: ethanol (1:19 v/v) and chloroform: ethyl acetate (80:20 v/v) respectively as shown in Figures (4-6).

DPPH Radical - Scavenging Activity: The results shown in Table 5 showed a comparison of the radical scavenging activity of flaxseeds with ascorbic acid that revealed radical scavenging activity of flaxseeds extract but less than standard ascorbic acid in all concentrations.

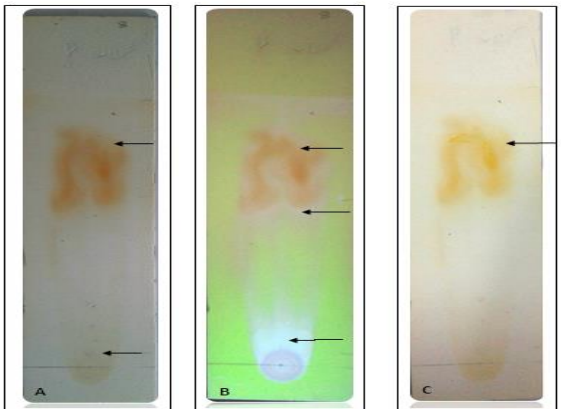

Figure 4: TLC of 20%methanolicextract of flaxseeds using developing solvent system ethyl acetate: ethanol (1:19 v/v). A: visible light. B: UV light. C: antioxidant band..

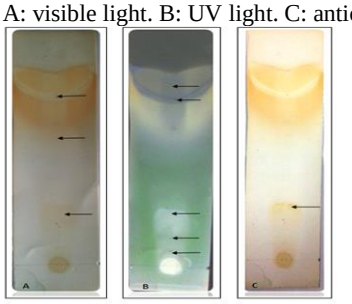

Figure 5. TLC of 20% methanolic extract of flaxseeds using developing solvent system chloroform :ethyl acetate (80:20 v/v). A: visiblelight. B: UV light. C: antioxidant band.

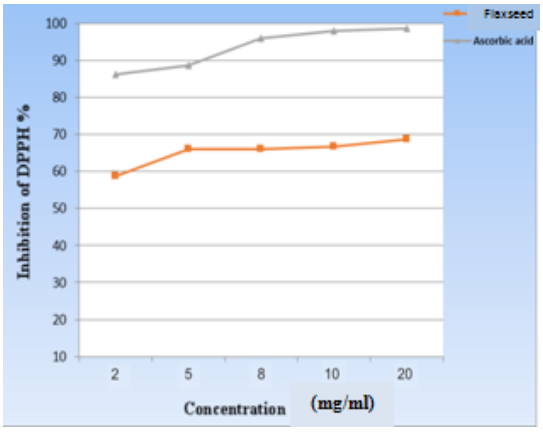

Figure 6: Percentage of inhibition of DPPH by 20% methanolic extract of flaxseeds as compared with standard ascorbic acid

Table 5: Scavenging activity of DPPH radical of 20% methanolic extract of flaxseeds and standard ascorbic acid

Concentration (mg/ml)	Scavenging activity of DPPH radical %	
	Ascorbic acid	Flaxseeds extract
2	86.19± 1.78a	58.75± 0.33b
5	88.7± 0.33a	65.98± 0.66b
8	96.04± 2.72a	66.1± 0.73b
10	96.80± 0.66a	66.67± 16.48B
20	98.55± 3.41a	68.72± 5.40b

Cytogenetic and Molecular Studies: Cytogenetic examination revealed that CPA effective in increased cell proliferation which estimated as a mitotic index. The results presented in Table 6 show that CPA group had a significant elevation (P≤0.05) in MI (2.10±0.15) compared to both D.W (1.2±0.05) and corn oil (1.3±0.02) controls. Flaxseeds extract 250 mg/kg showed nonsignificant differences (P>0.05) while flaxseeds extract 500 mg/kg made a significant increase (P≤0.05) in MI compared to DW control. Treatment with flaxseeds extract with CPA at two doses made a significant inhibition (P≤0.05) in the MI compared to CPA group which showed nonsignificant differences compared to control group.

Table 6: Changes in mitotic index (MI) of experimental rats treated with CPA and 20% methanolic extract of flaxseeds**.**

Groups	Mitotic index (MI) Mean ± S.E
Control (D.W)	1.20 ± 0.05abc
Control (corn oil)	1.3 ± 0.02 bcd
CPA 10 mg/ kg	2.10 ± 0.1e
Linseeds extract 250 mg/kg	1.45 ± 0.10Cd
Linseeds extract 500 mg/kg	1.63 ± 0.09D
CPA 10mg/kg+ linseeds extract 250mg/kg	0.93 ± 0.07 A
CPA 10mg/kg+ linseeds extract 500mg/kg	1.10 ± 0.19 ab

Chromosomal Aberrations: Tables 7 and 8 showed changes in the means and percentages of CA in bone marrow cells. CPA group recorded a significant increase (P≤0.05) in the CA percentage (7.2%) as compared to D.W. (2.2%) and corn oil (2.88%) control. In addition, treatment with flaxseeds alone or with CPA could not induce significant difference compared with both control groups but it significantly decreased (P≤0.05) when compared to CPA-treated group. Flaxseeds extract given with CPA in both doses had effect in reducing the higher percentage of chromosomal aberrations caused by CPA to (2.95 and 3.65) % in groups treated with CPA with flaxseeds 250 and 500 mg /kg respectively.

Table 7: Changes in some chromosomal aberrations of bone marrow cells of experimental rats treated with CPA and 20% methanolic extract of flaxseeds

Groups	Fragmented Chromosome Mean ± S.E	Chromatid break Mean ± S.E	Ring chromosome Mean ± S.E	Elongated chromosome Mean ± S.E	Aneuploidy Mean ± S.E	Dicentric chromosome Mean ± S.E
Control (D.W)	4.25 ± 0.73 a	7.25 ± 1.32 ab	0.00 ± 0.00 a	0.00 ± 0.00 a	10.50 ±0.50 b	0.00 ± 0.00 a
Control (corn oil)	6.50 ± 0.50 ab	9.00 ± 0.84 abc	0.00 ± 0.00 a	0.00 ± 0.00 a	13.25 ± 1.46 b	0.00 ± 0.00 a
CPA 10 mg/ kg	9.00 ± 1.45 c	23.75 ± 0.49 e	3.00 ± 0.84 c	4.25 ± 0.66 b	29.00 ± 0.71 d	3.00 ± 0.71 b
Linseeds extract 250 mg/kg	5.00 ± 0.84 a	10.25 ± 1.02 bc	2.00 ± 0.32 ab	3.25 ± 0.66 b	14.50 ±0.50 c	2.50 ± 0.74 b
Linseeds extract 500 mg/kg	3.75 ± 1.28 a	6.00 ± 0.32 a	3.00 ± 1.23 c	3.75 ± 0.66 b	6.25 ±1.07 a	3.00 ± 0.95 b
CPA 10mg/kg+ linseeds extract 250mg/kg	5.25 ± 0.66 a	10.50 ± 1.57 bc	2.00 ± 0.32 ab	0.00 ± 0.00 a	11.75 ± 1.28 b	0.00 ± 0.00 a
CPA 10mg/kg+ linseeds extract 500mg/kg	6.75 ± 1.02 Ab	12.00 ± 1.41 c	2.75 ± 1.2 c	1.00 ± 0.5 a	14.00 ± 1.52 c	0.00 ± 0.00 a

Table 8: Changes in percentage of some chromosomal aberrations of bone marrow cells of experimental rats treated with CPA and 20%methanolic extract of flaxseeds.

Groups	***Fragmented Chromosome %***	***Chromatid Break %***	***Ring Chromosome %***	***Elongated chromosome %***	***Aneuploidy %***	***Dicentric chromosome %***	***Sum %***
Control (D.W)	*0.425*	*0.725*	*0.00*	*0.00*	*1.05*	*0.00*	***2.2 a***
Control (corn oil)	*0.65*	*0.9*	*0.00*	*0.00*	*1.325*	*0.00*	***2.88 a***

CPA 10 mg/ kg	*0.9*	*2.375*	*0.3*	*0.425*	*2.9*	*0.3*	***7.2 b***
Linseeds extract 250 mg/ kg	*0.5*	*1.025*	*0.2*	*0.325*	*1.45*	*0.25*	***3.75 ab***
Linseeds extract 500mg kg	*0.375*	*0.6*	*0.3*	*0.375*	*0.625*	*0.3*	***2.58a***
CPA10mg/kg+linseeds extract 250mg/kg	*0.525*	*1.05*	*0.2*	*0.00*	*1.175*	*0.00*	***2.95 ab***
CPA 10mg/kg+ linseeds Extract 500mg/kg	*0.675* *4.05*	*1.2* *7.875*	*0.275* *1.275*	*0.1* *1.225*	*1.4* *9.925*	*0.00* *1.7*	***3.65ab***

DNA Fragmentation Test (DFT) of White Blood Cells and Testes Tissue: Figure 7 shows electrophoresis of DNA extracted from white blood cells and testes tissue of experimental groups. In CPA group, DNA has fragmentation level about 3200 bp in WBC and 6800 bp in test-es tissue compared with both control groups whi-ch have normal DNA band. Both groups treated with flaxseeds 250 and 500 mg/kg did not record any fragmentation in DNA of WBC while flax-seeds caused fragmentation of testes DNA in nor-mal rats which was 5800 and 3400 bp in 250 and 500mg/kg treated group respectively. In addition, treatment of flaxseeds with CPA could reduce the fragmentation level caused by CPA 10 mg/kg in both DNA of WBC and testes tissues which reached to 2400 and 2000 bp in 250 and 500 mg/kg of 20% methanolic extract of flaxseeds respectively in WBC while DNA extracted from testes of CPA group treated with 250 mg/kg had fragmentation level 4900 bp and 500 mg/kg dose was more effective in reducing fragmentation of DNA which was 3800 bp when given to CPA group treated for 50 days.

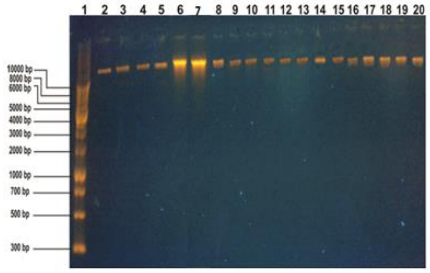

Figure 7: Level of DNA fragmentation of experimental groups

DISCUSSION

The result of different detection methods in this study revealed that the 20% methanolic extract of flaxseeds contained alkaloids, coumarins, essential oils, resins, phenolic compounds, saponins, flavones, steroids, and terpenes. Moreover, TLC screening showed the presence of different numbers of bands with different R_f value. This result indicates that 20% methanolic extract of flaxseed is a crude extract. it consistent with other studies which showed that the chemical analysis of the aqueous extract of flaxseeds contained alkaloids, coumarins, essential oils, resins, phenolic compounds, saponins, flavones, steroids, and terpenes while glycosides and tannins were absent (Monica and Joseph, 2016). Also, ethanolic extract of flaxseeds contains phenolic compounds, linoleic acid (11.8%), unsaturated fatty acid (73.8%) and total phytoesterol was 8.5%. Flaxseeds contain a valuable amount of omega-3 fatty acid, α-Linolenic Acid (ALA), dietary fiber, protein, lignan specifically Secoisolariciresinol diglucoside (SDG) (Al-Okbi *et al*., 2014; Gutte *et al*., 2015).

The present study revealed the presence of some antioxidant compound which indicates that flaxseeds had the antioxidant activity. One of the components of flaxseeds which has the effect of antioxidation is lignan that has a role in the scavenging for free radicals generated in the cell (Parasad, 2000).

The results of the cytogenetic study revealed a significant increase ($P \leq 0.05$) in MI of CPA group compared to both control D.W and corn oil. This result ensures that CPA has a has a mitogenic effect. The previous study detected that CPA could produce tumors in female rats. This impact ascribed to a rodent-specific tumor supporting a mode of action established on the finding out of a powerful hepatomitogenic activity of CPA and demonstrated that CPA is converted to intermediates which cause damage of DNA in hepatocytes of female rats that caused the forming of DNA adducts, gene mutations and elevation of micronuclei. Also, CPA could induce the forming of DNA adducts of human hepatocytes in primary cultures revealing that human hepatocytes have the ability to activate CPA to genotoxic intermediates (Kasper 2001).

In addition, CPA caused increasing in the cell proliferation in rat liver and the tumorigenic role may be due to growth-promoting effects (Schulte-Hermann *et al*., 1980).

The cytogenetic findings presented in Tables 7 and 8 revealed that elevation of CA may due to genotoxic effect of CPA. The CA arise from exposure to genotoxic agents that cause a break or exchange the chromosome pieces or chromosome number changes may cause cell death and others

cause genetic impacts in sex and somatic cells (Swierengo *et al*., 1991). These results are in concurrence with those established by Siddique *et al*. (2011) who recorded genotoxic damage produced by 30 µM of CPA in culture of lymphocytes which result in the significant increase in the frequency of SCEs /chromosomes.

Flaxseeds extract in both doses could reduce higher MI caused by CPA and revealed a significant decrease ($P \leq 0.05$) to (0.93±0.07) and (1.1 ±0.19) in groups treated CPA with flaxseeds 250 and 500 mg/kg respectively. This effect of flaxseeds due to an active phytochemical compound which may act synergistically to reduce the mitogenic effect of CPA. The major phytoestrogen groups in flaxseeds are isoflavones (genistein), flavones and lignans. Genistein could repress tyrosine kinases, that are dependable for phosphorrylating of proteins spesific for the controlling of cell functions, containing cell division. Therefore, it has been an exhibit to reduce growth in numerous cell lines (Peterson,1995).

These findings are in concurrence with the earlier study that showed the flaxseeds had effective antioxidant compounds as lignan which reduce CA in the cell (Parasad, 2000). Also, this effect of flaxseeds may due to active phytochemical compounds which may act synergistically to reduce higher MI and CA of the CPA-treated group. This result is in concordant with the previous study that assessed the antigenotoxic effect of flaxseeds against the genotoxic effect of cisplatin which induced statistically highly significant ($P \leq 0.001$) percentage of CA (9.2%) but flaxseeds significantly reduced this percentage to 4.6% (Al-Okbi *et al*., 2014). Also, chromosomal damage occurring in mice is prevented by flaxseeds lignin (Trentin *et al*., 2004) and it might be linked to the antioxidant potential of flaxseeds (Kangas *et al*., 2002). In addition, another finding indicated that flaxseed oil had protective effects and reduced the CA in bone marrow cells in a dose 0.1 ml /kg body weight in albino mice induced by anticancer drug methotrexate after injection intra-peritoneally at a dose of 10 mg/kg body weight. So flaxseed oil had protective role leads to lowering the CA that happened by Methotrexate (Othman, 2013).

Electrophoresis of DNA extracted from CPA group which had high fragmentation level of WBC and testes tissue may due to a potential role of ROS in the genotoxic potential of CPA (Siddique and Afzal, 2004). The production of ROS is responsible for the genotoxicity in cultured human peripheral blood lymphocytes and rats treated with CPA exhibit liver cytotoxicity (Ali, 2008). Flaxseeds contain numerous phytochemicals like flavonoids, cinnamic and phenolic acids, and lignins which are antioxidants and influence the growth of cell and viability. These phytochemicals defend against heart diseases and cancer (Arts and Hollman, 2005).

Both groups treated with flaxseeds 250 and 500 mg/kg caused fragmentation levels of DNA extracted from testes tissues which were 5800 and 3400 bp in 250 and 500 mg/kg treated groups respectively. This fragmentation of DNA may be due to the presence of some compound which has a negative effect on the genetic material of testes and was reflected as histological changes of testes and altered normal sperm parameters recorded in the previous study (Al-Harbi *et al*., 2017).

In addition, the reduction in the genotoxicity of CPA by 20% methanolic extract of flaxseeds may possibly because the effect of antioxidant compounds detected in this study by β-carotene and DPPH scavenger assays that could scavenge the ROS generated by CPA and thereby prevent the genotoxic damage of CPA. Flaxseeds are the richest origin of the lignan SDG which is a strong antioxidant (Petit *et al*., 2009; Adolphe *et al*., 2010). A larger body of evidence proposes that SDG metabolites can supply health advantage in consequence of their weak estrogenic or antioestrogenic impact, ability to inhibit or increase the activity of specific enzymes, antioxidant activity, or by mechanisms yet unknown. SDG metabolites can reduce inflammation and oxidative stress. Flaxseeds lignans can as well decrease the risk of cancer by impeding pre-cancerous cellular changes and by inhibiting metastasis and angiogenesis (Adolphe *et al*., 2010).

Conclusion

The present study shows the CPA 10 mg/kg had mitogenic and genotoxic effects while the 20% methanolic extract of flaxseeds having sufficient antimitogenic and antioxidant activities to reduce the genotoxic effects of CPA.

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