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Research Article

Composition and Genoprotective Effect of the Flavonoidal Content of *Lepidium sativum* L. Methanolic Seed Extract against Cyclophosphamide- Induced DNA Damage in Mice

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ABSTRACT

Background: *Lepidium sativum* L. (Family Brassicaceae) is known to possess different pharmacological properties. **Objective:** The genoprotective role of flavonoids of *L. sativum* methanolic seed extract (LSF) against cyclophosphamide (CP)-induced DNA damage, in somatic and germ cells of mice, as well as characterization of the flavonoidal content were carried out in this study. Chromosomal aberration analysis in somatic and germ cells were also included. **Materials and Methods:** Six mice groups were used for this study. Group 1 served as a negative control. Group 2 received oral LSF (100 mg/kg b.wt.) for 5 consecutive days. Group 3 served as a positive control by receiving a single intraperitoneal (i.p.) CP dose (20 mg/kg b.wt.). The three other groups were orally administered 25, 50 and 100 mg/kg b.wt. LSF, respectively, for 5 consecutive days. On the last day of treatment, the three groups received i.p. injection of CP (20 mg/kg b.wt.). Flavonoids were identified using spectral analysis. **Results:** LSF inhibited DNA aberrations in mice caused by cyclophosphamide dose dependently in the three groups with significant difference in the two groups that received doses of 50 and 100 mg/kg b.wt. The chromosomal aberrations inhibitory indices were calculated as 18 and 31 in mice somatic cells and 27 and 48 in germ cells, respectively. LSF was found to contain the flavonoids kaempferol, quercetin, kaempferol-3-*O*-α-L-rhamnopyranoside, kaempferol-3-*O*-β-D-glucopyranoside, and quercetin-3-*O*-β-D-galactopyranoside. **Conclusion:** LSF inhibited the DNA damage induced by CP in somatic and germ cells of mice dose-dependently. The antioxidant properties associated with flavonoids might account for the genoprotective activity.

Key words: Antioxidant activity, Chromosomal abberations, Flavonoids, *Lipidium sativum*.

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INTRODUCTION

Despite the great advances in modern medicinal scientific research, traditional medicine is still used in treating majority of diseases in developing countries. According to the World Bank in 1997, the significance and importance of medicines from natural origin have been increasing worldwide. Almost 50% of medicines present in the market all over the world are made of natural based materials. Medicinal herbs are likely to remain in high demand in the market due to the difficulty of synthetically preparing many active constituents of medicinal plants.[1]

Lepidium sativum L., also known as garden cress or garden pepperwort, is an important member of family Brassicaceae. It's a fast growing annual herb which is native to Egypt and west Asia but also widely cultivated in temperate climates.[2] *L. sativum* possesses many different medicinal properties.[1] The seed extract have been used in Indian traditional medicine since ancient times.[3] It also shows many medicinal properties such as anticancer,[4] antidiabetic, hypocholesterolemic,[5-7] antihypertensive,[8] antidiarrheal, antispasmodic, and laxative activities.[9-10] It also has fracture healing,[11] hepatoprotective,[12] diuretic,[13] as well as antimicrobial, anti-inflammatory, antipyretic and analgesic potentials.[14,15] It was also reported that *L. sativum* is effective in treatment of cough and bronchial asthma.[16]

Flavonoids exist in nature as diphenylpropanoids. In addition to their importance as an essential component in human diet, flavonoids were reported in literature to possess several pharmacological activities such as antioxidant, antiviral, anti-inflammatory and antitumor activities.[17] Flavonoids are also reported to be involved in preventing free radical-mediated cytotoxicity and lipid peroxidation, which are known to be associated with cell aging and chronic diseases.[18]

Human exposure to genotoxic agents has dramatically increased in the last decades. In our continuous interest in genoprotective constituents from natural origin,[19] the current study was carried out to investigate the safety and the possible genoprotective role of flavonoids of *L. sativum* methanolic seed extract (LSF) against the genotoxic effect of CP in bone marrow and spermatocyte cells of mice.

MATERIALS AND METHODS

Chemicals

Cyclophosphamide (CP) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used in extraction were purchased from ADWIC (Cairo, Egypt).

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Plant material

Lipidium sativum L. seeds were purchased from Egyptian market in February 2018.

Extraction, preparation of flavonoidal fraction (LSF) and isolation

The seeds of *L. sativum* were left at room temperature (35 ^{0}C) to remove any moisture until no loss of weight was detected. The air-dried seeds (350 g) were defatted with n-hexane then extracted with CH_2Cl_2 (3X2L) followed by MeOH till exhaustion at room temperature. The combined methanolic extract was dried at 40 ^{0}C under reduced pressure to yield a yellowish brown residue (22.2 g).The residue was dissolved in H_2O (0.2%), and the aqueous solution was passed through a 500 g chromatographic column packed with the polymer gel Diaion HP-20 (Mitsubishi, Tokyo, Japan). The column was then washed with distilled water for several times and elution was carried out using 25%, 50%, 75% aqueous MeOH followed by 100% MeOH. The collected fractions were examined by silica gel thin layer chromatography (TLC) (Merck, Darmstadt, Germany) using the solvent systems CH_2Cl_2-MeOH (5:1) and CH_2Cl_2-MeOH-H_2O (60:30:5) The fractions eluted from the HP-20 column with 50% and 75% aqueous methanol (5.1 g) were combined and proved to contain flavonoids (LSF) (TLC analysis and examination under UV light). The LSF fraction was kept in the refrigerator until use. Part of LSF (3.0 g) was subjected to silica gel column chromatography. The column was eluted with CH_2Cl_2 followed by CH_2Cl_2-MeOH with increasing amount of MeOH to 15%. A total of 35 fractions 25 ml each were collected. The fractions were monitored by silica gel TLC plates using solvent system CH_2Cl_2-MeOH (5:1) and examined under UV light followed by spraying with $FeCl_3$ reagent. Sub-fractions 4-7 (1.1 g) eluted by 5% aqueous methanol was subjected to preparative thin layer chromatography using CH_2Cl_2-MeOH (5:1) solvent system. The material from the observed two bands were repeatedly purified on Sephadex LH-20 column (25 g, Sigma) to yield kaempferol (15 mg) and quercetin (25 mg).The fractions eluted with 10%, 12% and 15% MeOH were combined (1.3 mg) and the combined fraction was subjected to repeated preparative silica gel TLC using solvent system CH_2Cl_2-MeOH-H_2O (60:30:5). The material from the three bands were repeatedly purified on Sephadex LH-20 column (25g) to yield kaempferol-3-*O*-*α*-L-rhamnopyranoside (15 mg), kaempferol-3-*O*-*β*-D-glucopyranoside (12 mg) quercetin-3-*O*-*β*-D-galactopyranosyl (10 mg). The isolated flavonoids identified by observing their color behavior under UV light and comparing their spectral data with the literature values (Figure 1).

Kaempferol (1)

Amorphous yellow powder, UV λ_{max} nm (MeOH): 254 sh, 268, 322 sh, 365; +NaOMe: 275, 320, 412; +$AlCl_3$: 262 sh, 270, 352, 426; +$AlCl_3$/HCl 260, 271, 350, 426; +NaOAc: 275, 300, 385; +NaOAc/H_3BO_3 269, 295 sh, 320 sh, 370. ^{1}H NMR (400 MHz, acetone-d6) δ: 12.04 (1H,S, OH-5), 8.02 (2H, d, J= 8.9 Hz, H-2', H-6'), 6.89 (2H,d, J=8.9 Hz, H-3', H-5'), 6.41 (1H, d, J=2.0 Hz, H-8), 6.14 (1H, d, J=2.0 H, H-6).

Quercetin (2)

Amorphous yellow powder, UV λ_{max} nm (MeOH): 258, 266 sh, 299 sh, 360; +NaOMe: 272, 327, 416, +$AlCl_3$: 275, 303 sh, 430; +$AlCl_3$/HCl 271, 300, 364 sh, 402, +NaOAc: 270, 325, 393; +NaOAc/H_3BO_3: 262, 298, 387. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.44 (1H,S, OH-5), 7.62 (1H, d, J= 2.0 Hz, H-2'), 7.50 (1H, dd, J=6.7, 2.0 Hz, H-6'), 6.85 (1H,d, J=6.7 Hz, H-5'), 6.36 (1H, d, J=1.5 Hz, H-8), 6.14 (1H, d, J=1.5 Hz, H-6).

Kaempferol-3-*O*-*α*-L-rhamnopyranoside (3)

Amorphous yellow powder, UV λ_{max} nm (MeOH): 265, 340, +NaOMe: 275, 388; +$AlCl_3$: 275, 300 sh, 347, 398; +$AlCl_3$/HCl: 275, 300 sh, 362; +NaOAc: 274, 300 sh, 362; +NaOAc/H_3BO_3: 262, 342. ^{1}H NMR (400 MHz, acetone-d_6) δ: 12.67 (1H, S, OH-5), 7.82 (2H, d, J= 8.4 Hz, H-2', H-6'), 6.99 (2H, d, J=8.4 Hz, H-3', H-5'), 6.43 (1H, d, J=2.3 Hz, H-8), 6.23 (1H, d, J=2.3 Hz, H-6), 5.49 (1H, d, J=1.5 Hz, H-1" Rha); 2.29-3.31 (4H, H-2"-H-5"), 0.86 (3H, d, J=5.6 Hz, Me-6"). ^{13}C-NMR (400 MHz, acetone-d_6) δ: 178.5 (C-4), 164.4 (C-7), 162.3 (C-5), 160.2 (C-4'), 157.7 (C-2), 157.2 (C-9), 134.9 (C-3), 130.8 (C-2',6'), 121.5 (C-1'), 115.5 (C-3',5'), 104.9 (C-10), 101.9 (C-1"), 98.8 (C-6), 93.7 (C-8), 71.3 (C-4"), 70.7 (C-3"), 70.6 (C-2"), 70.6 (C-5"), 16.9 (C-6").

Kaempferol-3-*O*-*β*-D-glucopyranoside (4)

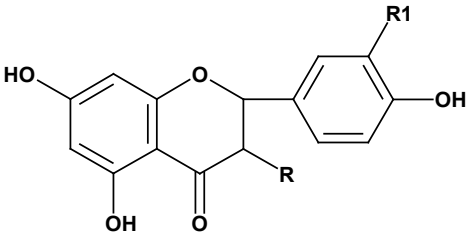
Amorphous yellow powder, UV λ_{max} nm (MeOH): 265, 300 sh, 351; +NaOMe: 273, 322 sh, 404; +$AlCl_3$: 275, 301sh, 345, 400; +$AlCl_3$/HCl: 273, 347, 402; +NaOAc: 271, 371; +NaOAc/H3BO3: 264, 351. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.60 (1H,S, OH-5), 8.04(2H, d, J= 8.8 Hz, H-2', H-6'), 6.88 (2H, d, J=8.8 Hz, H-3', H-5'), 6.33 (1H,d, J=1.8 Hz, H-8), 6.12 (1H, d, J=1.8 Hz, H-6), 5.43 (1H, d, J=7.3 Hz, H-1" Glc). 3.07-3.60 (sugar protons). ^{13}C-NMR (400 MHz, DMSO-d_6) δ: 177.55 (C-4), 161.60 (C-7), 160.42 (C-5), 157.03 (C-9), 156.29 (C-2), 133.54 (C-3), 131.27 (C-2',6'), 121.40 (C-1'), 115.56 (C-3',5'), 103.8 (C-10), 101.53 (C-1"), 99.69 (C-6), 94.38 (C-8), 77.93 (C-5"), 76.91 (C-3"), 74.69 (C-2"), 70.34 (C-4"), 61.30 (C-6").

Quercetin-3-*O*-*β*-D-galactopyranoside (5)

Amorphous yellow powder, UV λ_{max} nm (MeOH): 257, 268 sh, 363; +NaOMe: 273, 323 sh, 409; +$AlCl_3$: 272, 304sh, 437; +$AlCl_3$/HCl: 273, 348, 405; +NaOAc: 273, 324, 375; +NaOAc/H_3BO_3: 265, 298, 377. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.63 (1H,S, OH-5), 7.67 (1H, dd, J= 2.2, 8.5 Hz, H-6'), 7.53 (1H, d, J=2.2 Hz, H-2'), 6.83 (1H,d, J=8.5 Hz, H-5'), 5.38 (1H, d, J=7.6 Hz, H-1") 3.08-3.60 (sugar protons). 6.41 (1H, d, J=2.0 Hz, H-8), 6.20 (1H, d, J=2.0Hz, H-6). ^{13}C-NMR (400 MHz, DMSO-d_6) δ: 177.94 (C-4), 164.61 (C-7), 161.69 (C-5), 156.76 (C-9), 156.68 (C-2), 148.93 (C-4'), 145.29 (C-3'), 133.94 (C-3), 122.46 (C-6'), 121.55 (C-1'), 116.39 (C-5'), 115.64 (C-2'), 104.37 (C-10), 102.25 (C-1"), 99.13 (C-6), 93.96 (C-8), 76.30 (C-5"), 73.64 (C-3"), 71.66 (C-2"), 68.38 (C-4"), 60.59 (C-6").

Animals

Male white Swiss mice (*Mus musculus*), aged from 9 to 12 weeks were used in all experiments. The animals were obtained from a closed



	R	R_1
1	OH	H
2	OH	OH
3	*O*-*α*-L-rhamnopyranosyl	H
4	*O*-*β*-D-glucopyranosyl	H
5	*O*-*β*-D-galactopyranosyl	OH

Figure 1: Chemical structures of the isolated compounds.

random bred colony at the National Research Centre (Giza, Egypt). The mice used for each experiment were of similar age (±1 week) and weight (±2g). Animals were housed in polycarbonate boxes with steel-wire tops and bedded with wood shavings. Ambient temperature was controlled at 22±3 ºC with a relative humidity of 50±15% and a 12 h light/dark photoperiod. Food and water were provided *ad libitum*. The experiments were conducted according to the Animal Research Ethical Committee Guidelines of the National Research Centre.

Experimental design

A total of 30 mice were used for the experimental model. Mice were divided into six groups each consists of five animals. The animals were treated as follows:

Group 1 served as the non- treated, negative control. Group 2 received oral LSF by gavage (100 mg/kg b.wt.) for 5 consecutive days. Group 3 served as the positive control by receiving a single intraperitoneal (i.p.) dose of CP (20 mg/kg b.wt.). Groups 4, 5 and 6 were orally administered LSF at doses of 25, 50 and 100 mg/kg b.wt. respectively for 5 consecutive days. On the last day of treatment, the three groups received i.p. injection of CP at a dose of 20 mg/kg b.wt. Samples were collected 24 h after the last treatment.

Cytogenetic analysis

Chromosomal aberrations in somatic cells

Chromosomal preparations from bone marrow (somatic cells) were prepared according to Yosida and Amano method.[20] One hundred well-spread metaphases per mouse were analysed. Metaphases with gaps, chromosome or chromatid breakage, fragmentation, deletions, Robertsonian translocation, as well as numerical aberrations (polyploidy) were recorded.

Chromosomal abnormalities in germ cells

Spermatocytes (germ cells) chromosomal preparations were carried out according to the technique of Evans *et al.*[21] One hundred well-spread diakinase- metaphase I cells were analysed per animal for chromosomal aberrations. Metaphases with univalents, fragments, and chromosome translocations were recorded. Mice were injected i.p. with colchicine 2-3 h before animal sacrifice. Metaphases analysed under100X magnification with a light microscope (Olympus, Saitama, Japan).

Statistical analysis

The t-test was used to calculate the significance of the results of the chromosomal aberrations between the group which received the CP alone compared with those of the negative control group and the group which received CP with LSF. Statistical significance was taken as *p* values less than 0.01 for all experiments.

Evaluation of the activity of the LSF to reduce abnormalities induced by CP was carried out according to the following formula:[22]

Inhibitory index (II) = [1- (GCME plus CP – control) / (CP - control)] X 100.

RESULTS

Isolation and characterization of flavonoids

Five known flavonoids were isolated and characterized by spectroscopic techniques.

Chromosomal aberrations in bone marrow cells

The results in Table 1 showed the number and percentage of the chromosomal aberrations recorded for the animal treated groups: control, LSF, CP and LSF with CP in bone marrow cells (Figure 2). The percentage of aberrant cells in mice treated with LSF at 100 mg/kg b.wt. for 5 consecutive days (2.20±0.40) showed no significant difference from that of the negative control group (2.00±0.55). The results also showed the ability of the used doses of LSF to significantly reduce the aberrations induced by CP (*p>0.01*) in mice bone marrow cells in a dose dependent manner. The percentage of total abnormal metaphases were reduced from 23.80±0.45 in the group that received CP alone to 17.00±0.65 upon pretreatment with 100 mg LSF / kg b.wt. (excluding gaps).

Table 1: The inhibitory effect of flavonoidal content of the *Lipidium sativum* L. methanolic seed extract (LSF) on CP-induced chromosomal aberrations in mouse bone marrow cells *in vivo*.

Treatments (mg/kg b.wt.)	Total Abnormal Metaphases			No. of different types of metaphases						Inhibitory index Excluding Gaps (II)
	Number	Mean (%) ± SE		G.	Frag. and/ or Br.	Del.	C.F.	M.A.	Polyp.	
		Including Gaps	Excluding Gaps							
1. - ve Control	15	3.0 ± 0.40	2.00 ± 0.55	5	8	2	0	0	0	-
2. CP (20)	134	26.80 ± 0.50 [a]	23.80 ± 0.45 [a]	15	55	12	7	41	4	-
3. LSME (100)	19	3.80 ± 0.68	2.20 ± 0.40	8	9	2	0	0	0	-
4. LSME + CP a) 25	126	25.20 ± 0.70[a]	22.00 ± 0.54[a]	16	61	10	2	35	2	8
b) 50	112	22.40 ± 0.54 [ab]	19.80 ± 0.52 [ab]	13	58	7	1	31	2	18
c) 100	98	19.60 ± 0.50[ab]	17.00 ± 0.65[ab]	13	45	5	3	32	0	31

Total number of examined metaphases 500 (5 animals/group); G.: Gap; Frag.: Fragments; Br.: Breaks; Del.: Deletions; C.F.: Centric Fusions; M.A.: Multiple Aberrations; Polyp: Polyploidy. a: Significant compared to -ve control (*p<0.01*); b: Significant compared to CP treatment (*p<0.01*) (t-test).

Table 2: The inhibitory effect of flavonoidal content of the *Lipidium sativum* methanolic seed extract (LSF) on CP-induced chromosomal abnormalities in mouse spermatocyte cells *in vivo*.

Treatments (mg/kg b.wt.)	Total Abnormal Metaphases		No. of different types of metaphases					Inhibitory index (II)
	Number	Mean(%) ± SE	XY-uni.	Auto.uni.	XY-uni.+ Auto. uni.	Frag.	Chain (IV)	
1. - ve Control	14	2.80 ± 0.50	9	5	0	0	0	0
2. CP(20)	78	15.60 ± 0.55[a]	48	23	3	2	2	0
3. LSME (100)	16	3.20 ± 0.48	12	4	0	0	0	0
4. LSME + CP								
a) 25	69	13.80 ± 0.60[a]	46	15	4	1	3	14
b) 50	61	12.20 ± 0.50[ab]	34	24	1	0	2	27
c) 100	47	9.40 ± 0.55[ab]	30	13	1	1	2	48

Total number of examined metaphases 500 (5 animals/ group); XY-uni: XY- univalent; Auto. uni.: Autosomal univalent; XY-uni.+ Auto. uni.: XY-univalent + Autosomal univalent; Frag.: Fragment. a: Significant compared to -ve control ($p<0.01$); b: Significant compared to CP treatment ($p<0.01$) (t-test).

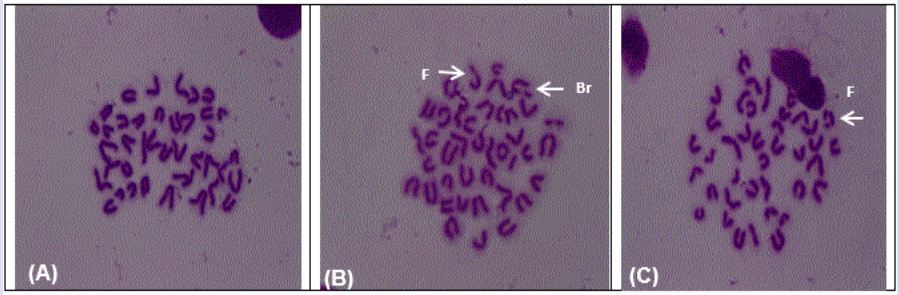


Figure 2: Chromosomal abnormalities in bone marrow cells in mice showing (A) normal, (B) fragment and break, and (C) fragment.

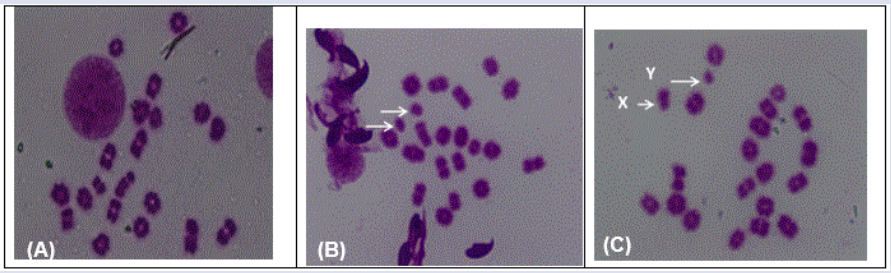


Figure 3: Chromosomal abnormalities in diakinase- metaphase 1 cells in mice showing (A) normal, (B) autosomal univalent and (C) XY univalent.

Chromosomal aberrations in spermatocyte cells

The effect of LSF on CP induced DNA damage in mice spermatocyte cells was determined by the percentage of chromosomal aberrations recorded in Table 2 (Figure 3). The percentage of chromosomal aberrations in spermatocyte cells showed significant elevation ($p<0.01$) in CP treated group compared to negative control group. Oral administration of LSF at the three different doses 25, 50 and 100 mg/kg b.wt. for 5 consecutive days reduced DNA damage induced by CP in a dose dependent manner. The inhibitory indices were 14%, 27% and 48%, respectively, with significant results ($p<0.01$) in the two groups treated with the highest two dose levels.

DISCUSSION

In the present study, five flavonoids were isolated after Diaion HP-20 and silica gel column chromatography from the methanolic seed extract of *L. sativum* L (Figure 1). The flavonoids were identified as kaempferol, quercetin, kaempferol-3-*O*-α-L-rhamnopyranoside, kaempferol-3-*O*-β-D-glucopyranoside, and quercetin-3-*O*-β-D-galactopyranoside by comparison of their spectral data with the literature values.[23-26] The flavonoidal content was then evaluated for its protective activity against genotoxicity induced by cyclophosphamide

Cyclophosphamide [N,N-bis(2-chloroethyl) tetrahydro-2H-1,3,2-oxphosphorin-2-amine, 2-oxide monohydrate], the pharmaceutical product also known as cytoxan or endoxan, is an antineoplastic agent which is used in treatment of a vast range of cancers such as leukemia, ovarian adenocarcinoma and some types of lung cancers. It is also used as an immunosuppressant agent in treatment of arthritis, systemic lupus erythematosus, multiple sclerosis, and organ transplantation.[27,28] Like many anticancer agents, CP fails to differentiate between normal cells and cancerous cells thus it causes the death of both.[29] Extensive studies showed that CP is a powerful mutagen and/or clastogen at all phylogenetic levels despite being a powerful chemotherapeutic agent. It causes dose related increase in genotoxic activities *in vivo* leading to the onset of different pathological conditions such as cancer. Such genotoxic activities were described as cross links and strand breaks in DNA, gene mutations, chromosomal aberrations, sister chromatid exchanges in addition to increased DNA adduct formation.[19,27,28,30]

L. sativum seeds are known for their nutritional importance and multiple health benefits.[18] The safety of the seeds was previously

evaluated in Wistar rats. Also, the acute and subchronic toxicity studies revealed that ingestion of *L. sativum* seeds for 14 consecutive days induced no toxic effects in rats in both cases.[31] In the present study mice groups intaperitoneally injected with 20mg/kg b.wt. CP showed statistically significant elevation ($p<0.01$) in the percentage of chromosomal abnormalities in both bone marrow and spermatocytes cells when compared to the negative control mice group (Figures 2 and 3). The results also showed that the daily oral treatment of mice for 5 consecutive days with LSF at a dose of 100mg/kg b.wt. caused non-significant effect in the percentage of chromosomal abnormalities in somatic and germ cells compared with the negative control group. Further, the animal groups which were orally administered LSF at the three dose levels 25, 50 and 100mg/kg b.wt. for 5 consecutive days followed by CP injection, showed a decrease in the percentage of DNA chromosomal aberrations in bone marrow and spermatocyte cells in a dose-dependent manner in comparison with the group injected only with CP. Mice groups treated with 50 and 100 mg/kg b.wt. showed significant inhibitory effects against DNA chromosomal aberrations with 18% and 31% in bone marrow cells as well as 27% and 48% in spermatocytes, respectively (Tables 1 and 2).

The effect of variable *L. sativum* extracts in reducing the damage caused by genotoxic chemicals was previously described. Mice treated with benzo(a)pyrene (BaP) after administration with *L. sativum* seed oil showed a decrease in germ cells genotoxicity which were indicated by a decrease in DNA damage, sperm abnormalities and lipid peroxidation level in addition to an increase in sperm counts, motility and GSH content. The results also showed that the seed oil improved the epididymal sperm quality, attenuated the testicular spermatogenic cell damage, restored the sperm counts and decreased the incidence of sperms with head abnormalities.[32] Also *L. sativum* juice was investigated for its chemoprotective activities against 2-amino-3-methyl-imidazo [4,5-f] quinolone-induced genotoxic effects and colonic preneoplastic lesions using single cell gel electrophoresis assays and aberrant crypt foci experiments, respectively.[33] Moreover, *L. sativum* juice was shown to protect against B(a)P-induced DNA damage in human derived cells and the effects were not attributed due to their content of isothiocyanates.[34]

The role of the flavonoids kaempferol, quercetin or their glycosides as genoprotective and antigenotoxic natural substances, was previously discussed. The antigenotoxic activity of quercetin was reported and attributed mainly to its ability to protect against oxidative stress and inhibiting enzymes responsible for bioactivation of genotoxic agents that caused major oxidative DNA injury.[35] Further biochemical studies suggested that DNA protective role of quercetin was based on enhancement of antioxidant defense system reduced glutathione (GSH) level, catalase (CAT) and glutathione peroxidase (GPx) activities with reduction in oxidative stress parameters such as thiobarbituric acid-reacting substance (TBARS), by deoperoxides and NO levels.[36,37] Kaempferol was shown to exhibit moderate antimutagenic effect. Glycosilation at C-3 showed no significant influence on glycosylated flavonoid antimutagenic activity.[38,39]

The antioxidant potentials of *L. sativum* seed extracts and their positive role in DNA repair system were previously discussed.[40] The free radical scavenging activity of the *L. sativum* seed methanolic extract was investigated and it was concluded that free radical scavenging activity of the extract may be attributed to the polyphenolic compounds which have the ability to scavenge free radicals and enhance the DNA repair system or DNA synthesis.[32,41] Also, Malar *et al.*,[42] showed that the ethanolic extracts of *L. sativum* seed extract exhibited high antioxidant activities and therefore form a potential source of natural antioxidant compounds.

Since CP was reported to cause oxidative stress in mice, increase the lipid peroxidation and decrease the anti-oxidant enzyme superoxide dismutase leading to generation of oxidative products, we concluded that the antioxidant properties of the LSF may account for its genoprotective activity.

CONCLUSION

In summary, the results of the present study showed that the flavonoidal content of *L. sativum* caused non-significant effect in the percentage of chromosomal abnormalities in somatic and germ cells. Moreover, it inhibited the DNA damage induced by CP in bone marrow cells and spermatocytes of mice in a dose dependent manner and this inhibition might be attributed to its antioxidant properties. The obtained results need further investigations to open the door for possible development of new drugs from natural origin.

CONFLICTS OF INTEREST

None.

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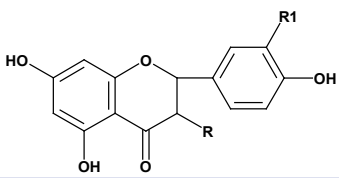
GRAPHICAL ABSTRACT



	R	R_1
1	OH	H
2	OH	OH
3	*O*-*α*-L-rhamnopyranosyl	H
4	*O*-*β*-D-glucopyranosyl	H
5	*O*-*β*-D-galactopyranosyl	OH

SUMMARY

Five known flavonoids were isolated from the methanolic seed extract of *Lipidium sativum*. Flavonoids from *L. sativim* inhibited the DNA damage induced by cyclophosphamide in somatic and germ cells of mice dose-dependently.

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