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# GENETICAL STUDIES OF FLAX SEED EFFECT ON LIVER AND SPLEEN OF RATS WITH NEPHROPATHY.



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## ABSTRACT

The flax seed is rich with lignans and α-linolenic acid plus antioxidant effects. The purpose of this study was to estimate the effect of flax seed on recovery of acute renal failure in rats. Twenty four rats were divided into four groups (each group has six individuals). One group was used as negative control (n=6), whereas the other three groups (n=18) injected with glycerol to induce acute renal failure. One of three groups fed on basal diet as positive control (C+), while the second and third groups (FS1 and FS2) was fed on 5 and 7% flax seeds for 28 days. In acute renal failure by glycerol in rats, kidneys, liver and heart weight was significantly increased when compared with negative control. RAPD amplifies the target genomic DNA with five primers generated a total of 116, 142 DNA fragments in liver and spleen, respectively. In liver, fifteen band of thirty eight (39.47 %) were polymorphic, whereas twenty three bands were monomorphic for all treatments. The highest level of polymorphism (55.56 %) was observed in primer 5 and the lowest level of polymorphism was 16.7% in primer 1. In spleen, total of bands resulted from five random primer was forty two bands, the polymorphic bands were sixteen (38.1 %) and the monomorphic band were twenty six bands (61.9 %).

**Keywords**: Flax seed, acute renal failure, DNA polymorphic.

## INTRODUCTION

The flax seed (*Linum usitatissimun* L., 2n = 30) considers a functional ingredient of great importance by the American Heart Association. It has functional combinations which made it very important, such as soluble fibers acid, linolenic and lignans that have a potential benefit to our health, especially to our heart, and recently cancer and liver failure (Prasad *et al.,* 1988; Samadi *et al.,* 2007). Flax seed is a rich source of lignans and plant α-linolenic acid which have been described to express beneficial effects in human health, lignans are platelet-activating factor-receptor antagonists, alpha-linolenic acid, omega-3 fatty acid, which has anti-inflammatory properties in addition to anti-atherogenic (Ingram *et al.,* 1995; Tarpila *et al*., 2005).

Flax seed has active components (lignans - secoisolariciresinol diglucoside - SDG) which have significant antioxidant effects where it inhibited the DNA's divisions and lipid peroxidation, also decreased production of reactive oxygen species. Lignans have numerous cardioprotective effects, these results supported by several clinical and experimental trials (Cerovic *et al.,* 2013).

Since NASH (nonalcoholic steatohepatitis) is strongly associated with metabolic syndrome, plus cardiovascular diseases, studying the functional properties of flax seed is extreme importance and enrichment for the scientific community and the population (Brea and Puzo 2013). Recently lignans have attracted attention as effective antioxidants in the context of relationship between diet and degenerative diseases such as cancer and cardiovascular diseases (ko *et al.,* 1995; Sung *et al.,* 2000 and Yu *et al.,* 2000). Flax seed contains high amounts of secoisolariciresinol diglucoside (SDG) (Muir and Westcott 2000) which has protective effects against hypercholesterolemic arteriosclerosis, diabetes, and cancer metastasis (Li *et al.,* 1999; Prasad 1999 and Prasad 2001). Thompson *et al.,* (1991) reported that dietary lignans can diminish the growth of both initial and advanced mammary tumors after studying the mechanism of it on the onset of rodent mammary cancer.

Different methodologies using molecular markers are widely used to analyse the pattern of variation within and between natural populations. Between the various marker systems, random amplified polymorphic DNA (RAPD) is one of the most famous DNA-based markers. It is the least technically demanding and offers a fast method for providing information from a large number of loci and it form the basis of novel genotoxicological assays for the detection of DNA damage and mutations. RAPD has proved valuable in many areas of biomedical research (Williams *et al*., 1990). RAPD profiles detect alterations to genomic DNA through the use of arbitrarily primed PCR reaction; these alterations include changes in DNA priming sequence sites and variations in the activity of the *Taq* DNA polymerase. This study aimed to apply RAPD-PCR to evaluate the effects of flaxseed on rat (2n= 42) with nephropathy using total genomic DNA of the liver and spleen.

## MATERIALS AND METHODS

**Rats and flax seeds**

Flax seeds were obtained from Cairo Haraaz market. Casein, vitamins, minerals and cellulose were obtained from EL- Gomhoria Company, Cairo, Egypt. Twenty four male albino rats (Sprague -Dawely Strain) were obtained from the laboratory of animal colony, Helwan farm.

**Experimental animals and conditions**

Male albino rats (n = 24) of Sprague Dawley strain weighing (190±10g) were housed in well aerated individual wire cages under hygienic laboratory conditions and fed on basal diet for one week for adaptation . Also, water was provided when needed.

**Experimental Diets**

The basal diet used in the experiment was prepared from fine ingredient pre 100 g as follows: casein 14 g (protein ≥ 80%), corn oil 4 g, cellulose 5 g, salt mixture 3.5 g, vitamin mixture 1 g, choline chloride 0.25 g and corn starch to complete hundred grams as described previously by Reeves *et* ***al.,*** **(1993**). Flax seeds were used at level 5 and 7% in the basal diet to prepare two experimental diets.

**Experimental design**

After the adaptation period, rats were divided randomly into two main groups; group (1) served as negative control ($C^-$) without glycerol injection since, six normal rats were fed on the basal diet. The main group (2) (n= 18 rats) were injected intramuscularly with glycerol in 0.9% saline at 10 ml/kg body weight to induce acute renal failure (Karam *et al.,* 1995). Where acute renal failure rats by glycerol was significantly higher as indicated in serum urea and creatinine levels. The subgroup (2) was divided into three subgroups (6 rats each). One of subgroup fed on basal diet as control positive (C+); the second subgroup (FS1) fed on 5% flax seeds diet whereas the third subgroup (FS2) fed on 7% flax seeds. All the above mentioned experimental groups were maintained on their corresponding diet for 28 days.

**Biological Evaluation**

During the experiment period (28 day), the consumed diet was recorded everyday (feed intake), and body weight was recorded every week. Biological evaluation of the different diets was carried out by determination of body weight gain % (BWG %) feed efficiency ratio (FER) according to Chapman *et al .,* (1959) as the following equation:

**BWG% = Final weight (gm) - Initial weight (gm)/Initial weight (gm).**

**Relative organs weight = Organs weight/Animal body weight× 100.**

At the end of the experimental period, rats were scarified under ether anesthetized. Liver, kidney and heart were removed from each rat, carefully washed with saline solution, dried with filter paper and weighted according to the method described by Drury and Wallington (1980), liver and spleen were taken for genetic studies.

**Total Genomic DNA Extraction**

Total genomic DNA was extracted by TIAamp genomic DNA Kit (Cat. no. DP304), (TianGen) according to the manufacture's protocol, rat tissue (10 mg) was re-suspended and centrifuged at 10.000 rpm for 1 min, then discard the flow-through and re-suspended cell pellet in 200 µl buffer GA, then 20 µl proteinase k was added and samples incubated at 56 Cº until the tissue is completely lysed, after that 200 µl buffer GB was added to the sample, mixed thoroughly by vortex and incubated at 70 Cº for 10 min, 200 µl of ethanol 96% was added to the sample and mixed by vortex for 15 second, the mixture pipet into the Spin Column and centrifuged at 12.000 rpm for 30 second, discarded the flow-through and added 500 µl buffer GD then centrifuged at 12.000 rpm for 30 second, the flow-through was discarded then added 600 µl buffer PW and centrifuged, discarded the flow-through, the Spin Column was placed in a new clean tube and pipet with 50-200 µl buffer TE, incubated in room temperature for 2-5 min, and then centrifuged for 2 min at

12.000 rpm. DNA was dissolved in 80 µl TE and became ready for PCR reaction

**Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR)**

Five RAPD primers were screened against the pooled rats DNA. The list of primers and their sequences are presented in Table 1.

**Table 1. Primers, their codes and sequences used in this study.**

| Number | Primer Code | Sequence (5`→3`) |
|---|---|---|
| 1 | GOM1-9 | `5`-CCCAAGGTCC- 3` |
| 2 | ISJ-1 | 5`-CAGACCTGCA- 3 |
| 3 | ISJ-3 | 5`-TGCAGGTCAG- 3` |
| 4 | ISJ-5 | 5`-CAGGGTTCCATCTGCA- 3 |
| 5 | ISJ-6 | 5`-ACTTACCTGAGCCAGCGA- 3 |

The optimization of PCR conditions for each primer was performed in a 20 µl reaction volume including 1 µl of isolated template DNA. Final concentration of each reaction was 1x master mix (MyTaq™ Red Mix, Bioline, England), 0.8 µM primer. Amplifications were carried out in a thermal cycler according to manufacture instructions as follow: the initial amplification program started with 95°C to denaturation for 2 min, followed by 35 cycles consisting of denaturation at 95°C for 15 s , annealing at 30°C for 20 s and elongation at 72°C for 1 min.

The program ended with a final elongation step at 72°C for 5 min. Amplification products were separated on 1.2 % agarose gel, stained with ethidium bromide and visualized under ultraviolet light. A known DNA Ladder (MassRuler DNA Ladder Mix ready-to-use, Cat-no: #SM0403 , Thermo Scientific) was run against the PCR products.

**Data Analysis**

The data were expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) was performed using the statistical package for social sciences (SPSS) software for windows version 20. Genomic template stability (GTS) is calculated by following equation: GTS (%) = (1 - a/n) ×100; where *a* is the number of polymorphic bands detected in each treated sample, and *n* is the number of total bands detected in the control. Polymorphism observed in RAPD profile included disappearance of a normal band and appearance of a new band in comparison to control RAPD profile (Luceri *et al.*, 2000; Atienzar *et al.*, 2002 and Qari 2010).

## RESULTS AND DISCUSSION

**Biological evaluation**

Table (2) showed the highest significant increase ($p<0.05$) of feed intake in control (-) followed groups treated with flax seeds (10.52±0.1, 9.17±0.5 and 9.11±0.4g) respectively, when compared with control (+). Groups treated with flax seed ($FS_1$) and ($FS_2$) seems to be effective for body weight gain (BWG%) and feed efficiency ratio (FER) if compared with control (+), they were significantly increase ($p<0.05$) of BWG% by 9.52±0.5 and

18.99±0.8, respectively, as well as, FER of treated groups by 0.105±0.3 and 0.109±0.6, respectively.

The relative weight of liver, kidney and heart to body weight was illustrated in the same Table. In acute renal failure by glycerol in rats, kidneys, liver and heart weight was significantly increased ($p<0.05$) when compared with negative control (-) of the group. All supplemented diet of injected rats by glycerol enhancement organs weight compared to control (+).

**Table 2. Feed intake, body weight gain (BWG%), feed efficiency ratio (FER) and organs weight/ body weight % for acute renal failure rats and treated groups (n=6 rats)**

| Groups | Feed intake (g/day) | BWG % | FER | kidney | liver | heart |
|---|---|---|---|---|---|---|
| Control(-) | 10.52±0.1$^{c}$ | 25.88±0.3$^{d}$ | 0.024±0.4$^{b}$ | 0.62± 0.05 $^{c}$ | 3.09±0.17 $^{d}$ | 0.34±0.03$^{c}$ |
| Control(+) | 6.43±0.2$^{a}$ | -30.65±0.4$^{a}$ | -0.046±0.2$^{a}$ | 1.25± 0.17 $^{a}$ | 4.6± 0.47 $^{a}$ | 0.57±0.05$^{a}$ |
| $FS_1$ | 9.11±0.4$^{b}$ | 9.52±0.5$^{b}$ | 0.105±0.3$^{c}$ | 1.17± 0.15 $^{b}$ | 4.17±0.18 $^{b}$ | 0.48±0.01$^{b}$ |
| $FS_2$ | 9.17±0.5$^{b}$ | 18.99±0.8$^{c}$ | 0.109±0.6$^{c}$ | 0.80± 0.07 $^{d}$ | 3.36±0.16$^{fc}$ | 0.35±0.02$^{c}$ |

**Mean± SD values, Means in the column with different letters are significantly different ($p \leq 0.05$).**

Previous studies evaluating effects of flax seeds on growth performance as Felmlee *et al.,* (2009), who suggested that the purified flax seed lignans, secoisolariciresinol diglucoside (SDG) and its aglycone metabolite (SECO) component of flax seed contributes to the hypocholesterolaemic and rate of weight gain effects. Also, Leite *et al.,* (2012) found that supplemented diet with flax seeds on rat milk creamatocrit and its contribution to offspring weight gain during lactation. Sacco *et al.,* (2011) reported that active components of flax seed provides protection against bone loss at the lumbar vertebrae primarily when combined with low-dose estrogen therapy in the ovariectomized rat model of postmenopausal osteoporosis and enhancement the liver, heart, kidney, thymus, and brain weights ($P < .001$) against total radioactivity in the skeleton. These data suggests that flax seeds increase BWG, FER, FI in rats with acute renal failure may be due to presence of protein, fatty acids (n-3 and 6 fatty acid), phytoestrogens, beta-glucan, total pentosanes, polyphenols and flavones, vitamin E, antioxidants and fibers (Report of analysis, 1995) leading to improvement the appetite and increasing feed intake.

**RAPD polymorphism among treatments in liver and spleen**

RAPD amplifies the target genomic DNA, with a short arbitrary primer (commonly 10 bp) in a PCR reaction, can be used to produce relatively complicated DNA profiles. Since the arbitrary primers complement different parts of the genomic DNA, PCR products will differ in number and size (polymorphism). In this study five primers (Table 1) were used to investigate the significant changes of DNA isolated from liver and spleen tissues of rats with nephropathy. The five primers as shown in Fig 1 generated a total of 116, 142 DNA fragments in liver and spleen, respectively.

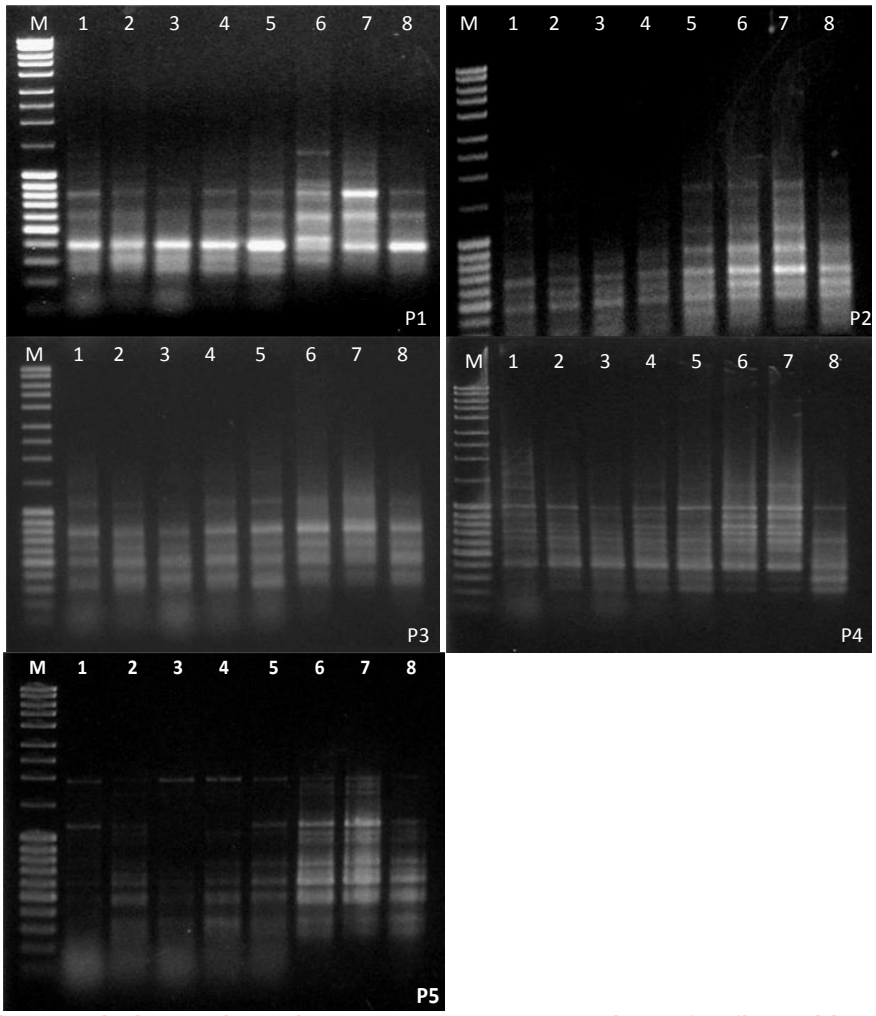


**Fig 1. Gel electrophoresis represents RAPD products for five arbitrary primers with isolated DNA from rat's liver and spleen.**

**M refers to the DNA ladder, 1 to 4 refers to DNA from Liver and 5 to 8 refers to DNA from spleen. 1 and 5 (negative control), 2 and 6 (positive control), 3 and 7 ($FS_1$), 4 and 8 ($FS_2$).**

Regarding liver DNA template, the results exhibited 116 amplified bands as a total number of the five primers application. Meanwhile, according to the band size, the obtained results showed 38 bands differ in its size. Fifteen bands out of them were polymorphic with the percentage of 39.47 %, whereas twenty three bands were monomorphic (common) for all treatments. The highest level of polymorphism (55.56 %) was observed with primer 5. Moreover, the lowest level of polymorphism was 16.7% with primer 1 as shown in Table 3.

**Table 3. Polymorphism level among treatments in liver if compared with control(-) via RAPD analysis.**

| Primer | TAF | PB | MP | P% | $Cont^-$ | $Cont^+$ | 5% | 7% |
|---|---|---|---|---|---|---|---|---|
| 1 | 6 | 1 | 5 | 16.7 | 6 | 5 | 5 | 5 |
| 2 | 5 | 2 | 3 | 40 | 5 | 3 | 3 | 3 |
| 3 | 5 | 1 | 4 | 20 | 5 | 5 | 4 | 5 |
| 4 | 13 | 6 | 7 | 46.15 | 11 | 7 | 9 | 10 |
| 5 | 9 | 5 | 4 | 55.56 | 4 | 9 | 6 | 7 |
| Total | 38 | 15 | 23 | 31.25 | 31 | 29 | 26 | 30 |

**TAF: Total Amplified Fragment, PB: Polymorphic Bands, MP: Monomorphic Bands, P%: Polymorphism percentage.**

Results from RAPD profile which appear in Table (4) refer to changes between control and other treatments, while control(-) showed in total 31 bands resulted from five primers, number of these bands ranged from four bands with primer 5 to eleven bands with primer 4. The positive control gives 12 variable bands (polymorphic bands include appearance of new bands and disappearance of normal bands), in addition to 12 and 11 for $FS_1$ and $FS_2$ flax seed respectively, if compared to the negative control.

**Table 4. Changes in DNA-RAPD profile in rat's liver with nephropathy feeded with flax seed at dosage of $FS_1$ (5%) and $FS_2$ (7%).**

| Primers | No. of bands in control (-) | Control(+) | | | | $FS_1$ | | | | $FS_2$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | a | b | c | d | a | b | c | d | a | b | c | d |
| P1 | 6 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 2 | 0 | 1 | 0 | 0 |
| P2 | 5 | 0 | 2 | 1 | 0 | 0 | 2 | 1 | 0 | 0 | 2 | 1 | 0 |
| P3 | 5 | 0 | 1 | 2 | 0 | 0 | 1 | 2 | 1 | 0 | 1 | 2 | 0 |
| P4 | 11 | 2 | 4 | 0 | 0 | 2 | 4 | 0 | 1 | 2 | 3 | 0 | 0 |
| P5 | 4 | 2 | 0 | 2 | 2 | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 1 |
| Total | 31 | 4 | 8 | 5 | 3 | 3 | 9 | 3 | 4 | 4 | 7 | 3 | 1 |
| a+b | | 12 | | | | 12 | | | | 11 | | | |
| GTS % | | 61.3 | | | | 61.3 | | | | 64.5 | | | |

**a: appearance of new band, b: disappearance of normal band, c: increase in band intensity, d: decrease in band intensity, a+b: polymorphic bands, GTS: genomic template stability**

The results appeared high increase in band intensity in positive control if compared with negative control and other treatments, which record 5 bands follow by $FS_1$ and $FS_2$ flax seed treatment since they recorded 3 bands for both treatments. On the other hand, the decrease in intensity of bands was 3 bands in the positive control whereas $FS_1$ flax seed recorded decrease in 4 bands intensity compared with $FS_2$ flax seed which decreases to one band. In this study, the results showed that the increase in polymorphic bands (15) and intensity in positive control in rats with renal failure which induced by glycerol decrease obviously in treatments with flax seed ($FS_2$). This indicates that it has high role in decrease side effects presence in rat renal failure or by another meaning, decrease the side or toxic effects of glycerol.

In spleen, total of bands resulted from five random primers was forty two bands (Table 5), the polymorphic bands were sixteen (38.1 %) and the monomorphic bands were twenty six bands. Most of the modifications occurred in the positive control and $FS_1$ were recorded with high value in polymorphic bands, in addition to band intensity increasing. On the other hand, flax seed $FS_2$ was obviously low in these changes. It can conclude that concentration of $FS_2$ decrease the damage in spleen. Furthermore, the increasing intensity was observed in both control (+) and treatment $FS_1$ flax seed, whereas the intensity in $FS_2$ was very low. No change in decreasing intensity was observed in the positive control and treatments.

**Table 5. Polymorphism level among treatments in spleen compared with negative control via RAPD analysis.**

| Primer | TAF | PB | MP | P% | $Cont^-$ | $Cont^+$ | 5% | 7% |
|---|---|---|---|---|---|---|---|---|
| 1 | 8 | 5 | 3 | 62.5 | 5 | 7 | 4 | 3 |
| 2 | 7 | 2 | 5 | 40 | 7 | 7 | 7 | 5 |
| 3 | 5 | 0 | 5 | 0 | 5 | 5 | 5 | 5 |
| 4 | 10 | 5 | 5 | 50 | 10 | 10 | 10 | 5 |
| 5 | 12 | 4 | 8 | 33.33 | 8 | 12 | 12 | 10 |
| Total | 42 | 16 | 26 | 37.17 | 35 | 41 | 38 | 28 |

**TAF: Total Amplified Fragment; PB: Polymorphic Bands; MP: Monomorphic Bands; P%: Polymorphism percentage.**

**Table 6. Changes in DNA-RAPD profile of rat's spleen with nephropathy feeded on flax seed at dosage of $FS_1$ (5%) and $FS_2$ ( 7%)**

| Primers | No.of bands in control (-) | Control(+) | | | | FS1 | | | | FS2 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | a | b | c | d | a | b | c | d | a | b | c | d |
| P1 | 5 | 3 | 1 | 2 | 1 | 0 | 2 | 2 | 1 | 0 | 2 | 0 | 1 |
| P2 | 7 | 1 | 0 | 2 | 0 | 1 | 1 | 2 | 0 | 0 | 2 | 0 | 1 |
| P3 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| P4 | 10 | 1 | 1 | 3 | 2 | 1 | 1 | 4 | 2 | 0 | 5 | 0 | 0 |
| P5 | 8 | 3 | 0 | 6 | 0 | 3 | 0 | 6 | 0 | 0 | 0 | 5 | 1 |
| Total | 35 | 8 | 2 | 13 | 3 | 5 | 4 | 14 | 3 | 0 | 10 | 5 | 3 |
| a+b | | 10 | | | | 9 | | | | 10 | | | |
| GTS % | | 71.43 | | | | 74.29 | | | | 71.43 | | | |

**a: appearance of new band, b: disappearance of normal band, c: increase in band intensity, d: decrease in band intensity, a+b: polymorphic bands, GTS: genomic template stability**

**Comparison of treatments with genome template stability (GTS):**

Genomic template stability (a qualitative measure reflecting the obvious changes in RAPD profile to the number of DNA bands in DNA patterns generated by flax seed with nephropathy rats) in relation to profiles obtained from control animals. GTS calculated with five primers was presented in Tables 4 and 6. Changes in RAPD profiles were expressed as reduction in GTS with values 61.3, 61.3 and 64.5 % in positive control, $FS_1$

and $FS_2$ respectively, in the rat liver (Table 4). In spleen, the GTS recorded 71.43, 74.29 and 71.43, respectively (Table 6). This result exhibited a harmony with those reported by Al-Bishri (2013), who demonstrated the first time the favorable effects of a flax seed-supplemented standard diet in improving liver and kidney functions in the hypertensive condition. These effects are likely to be mediated by the alpha-linolenic acid (ALA) and linoleic acid (LA) contents of flax seed oil due to its demonstrated ability to lower the blood pressure. Moreover, Silva *et al.* (2013) and Hall *et al.* (2006) suggested that flax seed protein isolated and hydrolysates have potential functional food ingredients with antioxidant capacity. This positive effect of flax seed may be resulted from antioxidant in flax seed. Flax seed has some dietary antioxidants, such as condensed tannins and flavonoids, which may also contribute the health benefits. The positive effect of flax seed may be resulted from secoisolariciresinol diglycoside whereas, Thompsona *et al.* (1996) demonstrated that flax seed has antitumorigenic effects, since secoisolariciresinol diglycoside (SD) was isolated from flax seed and tested for effects on mammary tumorigenesis in rats fed a high-fat (20%) diet. Their results showed that SD has an antitumor effect when provided at the early promotion stage of tumorigenesis and may contribute to the health benefits of high fiber foods. These results also agreed with Endoh *et al.* (2002), who reported that pretreatment of flax seed extract reduced extent of the necrosis found 24 hr after the intraperitoneal administration of CCl(4). Pretreatment of flax seed extract protect against CCl(4)-induce decrease of reduced glutathione-content measured from reactions with 5,5'-dithiobis-(2-nitrobenzoic acid) and also protect against the elevation of DNA strand breaks in the liver cells measured by comet assay. Flax seed-extract appears to protect liver cells against CCl(4)-induced necrosis. Flax seed oil is a natural product can protect against lead acetate-mediated hepatic cytotoxicity. Flax seed can inhibit colon carcinogenesis in cell cultures and animal models, and this preventive effect might be attributed to the high level of ω-3 polyunsaturated fatty acids, dietary fibers, and lignans, (Bommareddy *et al.* 2006 and 2009; Abdel-Moneim *et al.* 2011).

In conclusion, variation in band intensity, disappearance of normal bands and appearance of new bands occur in RAPD profiles for treatments were applied. This study aimed to apply RAPD-PCR to evaluate the effects of flax seed on rat with nephropathy using total genomic DNA of liver and spleen.

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## دراسات وراثية على تاثير بذور الكتان على كبد وطحال فئران مصابة بالفشل الكلوى

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تعتبر بذور الكتان غنية باللجنين، الفا حامض اللينوليك بالاضافة الى تأثيرات مضادات الأكسدة. تم تقسيم 24 فأر الى أربع مجاميع (كل مجموعة تحتوى على ستة أفراد). استخدمت مجموعة منها ككنترول سالب (ن=6) بينما الثلاث مجموعات الاخرى (ن=18) حقنت بالجليسرول لاحداث فشل كلوى حاد بها. مجموعة من هذه الثلاث مجموعات تم تغذيتها على وجبات تغذية أساسية واستخدمت ككنترول موجب (+C) بينما تم تغذية المجموعة الثانية والثالثة (FS1, FS2) على 5 ،7 % من بذور الكتان لمدة 28 يوم. فى حالة الفشل الكلوى الحاد بواسطة الجليسرول فى الفئران، لوحظ زيادة معنوية فى وزن الكلية ، الكبد ، القلب عند مقارنتها بالكنترول السالب. تم استخدام تكنيك ال RAPD لمضاعفة ال DNA الخلوى بواسطة خمسة بريمر ونتج عن ذلك 116 ، 142 حزمة DNA فى الكبد والطحال على الترتيب. وجد فى الكبد، 15 حزمة DNA من بين 38 حزمة بنسبة (39.47 %) متعددة الشكل المظهرى، بينما 23 حزمة وحيدة الشكل المظهرى فى كل المعاملات. تم مشاهدة أعلى معدل من تعدد الشكل المظهرى (55.56 %) مع البريمر رقم 5 بينما أقل معدل من تعدد الشكل المظهرى كان 16.7 % مع البريمر رقم 1. فى الطحال، كان اجمالى حزم ال DNA الناتجة مع الخمس بريمر حوالى 42 حزمة، كانت القطع التى أظهرت تعدد الشكل الظاهرى حوالى 16 قطعة (38.1 %) بينما وحيدة الشكل المظهرى كانت 26 حزمة (61.9 %).