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Study the effect of extra virgin olive oil in ameliorating the deleterious effects of soya bean on some biochemical parameters, antioxidant enzymes, DNA and pathological changes in male rats.

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Abstract

The purpose of this study is to determine the effect of extra virgin olive oil in ameliorating the deleterious effects of soya bean on some biochemical parameters, antioxidant enzymes, DNA, and pathological changes in rats. Four groups of male rats (10rats/group) were used. Group (1) was served as a control and fed on the basal ration only, group (2) fed on the basal ration mixed with soya bean (15%), group (3) fed on the basal ration mixed with olive oil (5%), and group (4) fed on the basal ration mixed with soya bean (15%) and olive oil (5%).The experimental period was 30 days.

At the end of the experiment, blood samples were collected from each rat for some biochemical analysis and oxidative and antioxidative parameters (malondialdehde MDA and glutathione s- transferase, GST). Rats were sacrificed then liver and kidneys samples were taken for studying DNA fragmentation and histopathological changes. Results of soya bean supplemented group (G 2) exhibited significant decreased of GST and increase in MDA, HDL, liver enzymes, urea, creatinine, total protein, albumin and globulin levels comparing with control group.

Supplementation of Extra virgin olive oil (G 3) resulted in significant increased of GST , total protein, globulin comparing with control group. Addition of extra olive oil to soya bean (G 4) improved the alteration of some parameters as MDA, GST, HDL, LDL, liver enzymes, urea, creatinine, total protein, albumin, globulin. Moreover, Olive oil had beneficial role in modulating the adverse effects of soya bean on DNA and some pathological changes.

Key words: *extra virgin olive oil, soya bean, biochemical parameters, antioxidant enzymes, DNA, pathological changes*

Introduction

Plant foods not only represent the major source of nutrients for human, but also contain protective factors against chronic diseases as coronary heart disease, diabetes and cancer. Plants contain some or all of the following characteristics: rich sources of antioxidant vitamins (B_1, B_2, C, alpha-tocopherol and beta-carotene); rich in fiber and high in protein **(Simopoulus 2001).**

Soyabean is a species of legume native to East Asia, widely grown for its edible bean which has numerous uses. The plant is classed as an oilseed rather than a pulse by Food and Agricultural Organization (FAO). It contains the isoflavones: genistein and daidzein, types of phytoestrogen **(Faqi, *et al.,* 2004)** that are considered by some nutritionists and physicians to be useful in the prevention of cancer **(Gardner, *et al.,* 2003)** and by others to be carcinogenic, embryotoxic, teratogenic and endocrine disruptive **(Xing, L. *et al.,* 2010).** Genistein (GEN) which is the most abundant soy isoflavones, reportedly can selectively bind

to the estrogen beta receptor and exert both estrogenic and antiestrogentic activity **(Mc Carty., 2006)**. Supplementation of GEN to neonatal mice produced a spectrum of effects similar to those reported for estrogenic endocrine disruptors, EEDs **(Padilla-Banks, *et al.,* 2006 and Jefferson, *et al.,* 2007)**. EEDS have the potential to produce wide spread adverse effect as carcinogenicity, neurotoxicity, immunotoxicity, interferance with the cardiovascular system, reproductive abnormalities and developmental toxicity **(Xing, L. *et al.,* 2010)**. Furthermore, **Lephart, *et al.,* (2001)** recorded that dietary soy phytoestrogen has significantly decrease body prostate weights and significantly change during adulthood in the structure of the sexually dimorphic brain region in male, but not in female rats. In the same line**, Mc Clain, *et al.,* (2005)** reported that treatment with genistein induced atrophy of testes and absence of spermatozoa in the epididymus of Beagle dogs. Moreover, soya beans grits and flour produced fetal morphological, visceral and skeletal malformations **(Maha, Z.G. 2011)**.

High protein diets are suspected to be detrimental to the renal and hepatic functions, calcium balance and insulin sensitivity, such as increased urinary nitrogen excretion, glomerular filtration rate, kidney hypertrophy, renal hemodynamics and eicosanoid production in renal tubules **(Bankir and Kriz, 1995 and Yanagisawa and Wada, 1998)** and increased risk of renal cell cancer **(Chow, *et al.,* 1994)**. **Gu, C. and Xu, H. (2008)** investigated that ingestion of high protein diets could result in an imbalance between oxidant and antioxidant, and thus induce oxidative stress in digestive organs of mice.

Olive oil is a fruit oil obtained from the olive (Oleaeuropaea). Olive oil is considered healthy oil because of its phenolic, oleic, palmitic acids, other monounsaturated fatty acid, vitamin E and carotenoids **(Elham *et al.,* 2008).** Leaves of olive trees contain large amounts of potentially useful phytochemicals and could play beneficial roles in health care and has antigenotoxic effect **(Anter *et al.,* 2011)**. The major phenolic compounds identified and quantified in olive oil belong to three different classes: simple phenols (hydroxytyrosol, tyrosol); secoiridoids (oleuropein, the aglycone of ligstroside, and their respective decarboxylateddialdehyde derivatives) and the lignans (1-acetoxypinoresinol andpinoresinol). All these phenolic substances have potent antioxidant properties as ascavengers of oxygen free radical **(Tuck and Hayball, 2002)** and protect colon, breast and skin against cancer, coronary heart disease, and ageing by inhibiting oxidative stress.

The previous result recorded by **(Machowetz, *et al.,* 2007)**, they showed that adequate supplementation of olive oil protect DNA against oxidative damage and reduced cancer incidence and may help in preventing the oxidation of LDL particles and affected on cholesterol regulation **(Covas, 2007)**.

On the basis of the above consideration, the aim of our study was to evaluate the benefit effects of extra virgin olive oil in ameliorating the possible undesirable effects of soya bean on some serum biochemical parameters, antioxidant enzymes, DNA and pathological changes in male rats.

Materials and Methods

I. Materials

(A)-Plants.

1-Soya bean: Soya bean was obtained from Faculty of Agriculture, Cairo University. .It was ground, freshly prepared and mixed with the basal diet of rats at a concentration of 15% according to **Olusola, *et al.,* (2011).**

2-Olive oil (virgin olive oil): was obtained from National Research Center, Cairo. It was freshly prepared and mixed with the basal diet of rats at a concentration of 5% according to **Kasdallah-Grissa, *et al.,* (2008).**

(B)-Animals.

Forty apparently healthy mature white albino male rats of an average body weight 150-180 g were used .They were obtained from the animal house of Ophthalmic Research Institute, Giza. The animals were acclimatized to the laboratory condition before using. Basal diet was formulated to cover all essential vitamins

and trace elements requirements .Food and water supply were given ad-libitum.

II. Methods.

1-Experimental design.

Forty mature male rats were divided into (4) equal groups (10 rats /group) as follows:

1. Group 1 (G1) served as control, fed on basal diet.
2. Group 2 (G2) was fed basal diet mixed with soya bean (15g /100g ration).
3. Group 3 (G3) was fed basal diet mixed with olive oil (5g /100g ration).
4. Group 4 (G4) was fed basal diet mixed soya bean (15%) and olive oil (5%).

2- Sampling.

(a)-Serum samples: At the end of the experiment blood samples were obtained from each animal group from orbital plexuse and received into clean dry tubes. Samples were left to clot at room temperature, and centrifuged at 3000 r.p.m. for 15 min. Serum samples were drawn in dry clean capped bottles and kept in a deep freeze for estimation of some biochemical and oxidative and antioxidative parameters.

(b)-Tissue samples : Animals were sacrificed and samples from liver and kidneys of each animal were obtained for DNA fragmentation and histopatholical examination.

3- Assays.

a- Histopathological examination :

1. Postmortem examination was done immediately after rats slaughtering.
2. Tissue specimens from liver and kidneys were collected and fixed in 10% neutral buffered formalin. They were routinely processed by standard paraffin embedding technique. Section at 4 micron, stained with Hematoxylin and Eosin **(Bancroft and Gamble, 2002).**

b- Some serum biochemical analysis:

The activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of **Reitman and Frankel (1957).** Alkaline phosphate (ALP) was determined according to **Dumas, *et al.*,(1971).** Serum urea and creatinine were estimated according to method of **Reises, *et al.*, (1965)** and **Faulkner and King (1976)**, respectively. Triglyceride was adapted by **Wahlrfeld (1974)**. Serum total cholesterol was determined according to method of **Richmond (1973),** HDL (high density lipoprotein) according to method of **Assmanm, (1979)** and LDL (low density lipoprotein) according to method of **Bachorik and Ross (1995**). Serum glucose was assayed according to **Trinder (1969)**. Estimation of serum total protein and electrophoretic pattern were carried out after **Sonnenwirth and Jaret (1980)** and **Davis (1964).**

c - Oxidative and antioxidants parameters: Estimation of lipid peroxide (MDA): was determined according to **Ohkawa, *et al.*, (1979)**. Estimation of glutathione s-transferase (GST) was assayed according to **Tamura, *et al.*, (1982)**.

d - DNA LADDER ASSAY : The extent of DNA fragmentation (DNA ladder) has been assayed by electrophoresing genomic DNA.

Samples were isolated from normal as well as treated rats liver, on agarose / ethydium bromide gel by the procedure described by **Sellins and Cohen (1987).**

e -Statistical analysis : Parametric data were statistically analyzed using Analysis Of Variance (ANOVA) test comparative of means were performed according to **Duncan (1955)** using **SSPSS 14 (2006).**

Results and Discussion

A- Effect on some biochemical parameters.

Data of serum samples obtained from animals received soya bean and /or olive oil were depicted in tables 1, 2, 3 and 4 .

In the present study rats fed on ration mixed with soya bean (G2) exhibited a significant increase MDA level (indices of lipid peroxidation) and significant decrease GST comparing with other groups (table 1). Our results in accordance with **chunmei *et al.*, (2008)**, they found that high protein diets lead to increase in MDA level (the precursor of most reactive oxygen species ROS) and a mediator in oxidative chain reactions. In addition high protein diets increased amino acid oxidation and urea synthesis **(Price, *et al.*, 1994)** and decreased the nutritional efficiency of energy utilization

(Porrata, *et al.,* 1987 and Porrata, *et al.,* 1990). However, the reoxidation of reducing equivalents derived from amino acid oxidation is linked to the mitochondrial redox chain **(Petzke and Proll 1994)**. Free radical generation during mitochondrial oxygen reduction may lead to oxidative stress if the antioxidant capacity is insufficient to quench the extra free radical production.

Moreover the increase in MDA level indicate that free radicals formation are accelerated and the cell membrane underwent peroxidation and in turn MDA increased and GST which responsible for detoxification was depleted and cell damage occurred **(Gokhan *et al.,* 2005)**.

Soya bean supplemented rats (G2) showed insignificant changes in cholesterol, LDL and triglyceride values but HDL level was significantly increased comparing with control group and other groups table (2).

Our results were in agreement with results documented by **Anthony *et al.,* (1996)** and **Yousef, *et al.,* (2004)**, they said that phytoestrogen-containing soya reduced serum LDL and VLDL cholesterol concentrations and increased serum HDL concentrations. The previous results may be attributed to that soya proteins interact with the saponins to form insoluble complexes, and thus it causes hypocholesterolemic actions **(Potter *et al.,* 1993)**.

The results in (Table 3) showed that soya bean alone(G2) caused significant increase in serum AST, ALT and ALP activities, as well as urea and creatinine concentrations but glucose level did not show any change comparing with other groups. Our data in accordance with **Chao *et al.,* (2002)** and **Anosike, *et al.,* (2008)** ,they reported that rats fed on frying soya bean recorded significant increase in AST, ALT and ALP levels. These elevations may be due to slight hepatic necrotic foci and hypercellualarity of renal glomeruli **(Kosif *et al.,* 2010)**.

In the same manner **Yousef, *et al.,* (2004)** found that treatment with isoflavones caused significant increase in serum AST,ALT and ALP activities.

Concerning with serum protein values our study as represented in table (4), showed that soya bean given to rats (G2) induced increase values in serum total protein, albumin and total globulin. These results were in agreement with the previous study recorded by **Rekowt, *et al.,* (1989)**, they found that high protein diet represent in soy products increase total protein , albumin and globulin than those fed lower protein diets.

Rats fed ration mixed with olive oil (G3) showed significant increase in GST level and insignificant decrease in MDA when compared with other groups and these results due to the phenolic substances which present in the olive oil which have potent antioxidant properties as scavengers of oxygen free radical according to **(Tuck and Hayball, 2002)**.

In the present study feeding rats with ration mixed with olive oil (G3) showed significant decrease in HDL concentration comparing with (G2) and insignificant changes comparing with other groups (G1, G4), while cholesterol, LDL and triglyceride not show any significant changes comparing with other groups. Results in table (1,3) showed significant decrease in MAD, AST,ALT , ALP, urea and creatinine concentration and a significant increase in GST level in G3 comparing with soya bean supplemented group. In addition, Levels of total protein, albumin and globulin were significantly decreased in G3 compared with G2.

The present investigation revealed that feeding rats ration mixed with soya bean and olive oil (G4) showed significant improvement in most biochemical, oxidative and anti-oxidant parameters when compared with soy bean group (G2) and control rats (G1). These results might be explained by **Kasdallah-Grissa *et al.,* (2008)**, **Violante *et al.,* (2009)** and **Lomborg *et al.,* (2008)**, they reported that diet contains olive oil induced reduction of lipid profile, increased antioxidant activity and decrease levels of liver enzymes. Moreover, **Lopez *et al.,* (2004)** reported that the antioxidant activity of extra virgin olive oil as a scavenger of free radical may be due to mainly phenolic compounds (hydroxytyrosol and tocopherol) which can also minimize the amount of reactive oxygen species(ROS) generated by fatty acids per-

oxidation and reducing liberation of oxidative enzymes.

B- Effect on DNA fragmentation and damage.

The extent of DNA fragmentation (DNA ladder) has been assayed by electrophoresing genomic, DNA samples were isolated from rat liver as showed in fig.(1) .

We noticed that the degree of DNA fragmentation is not observed in G1(control) and G3 (olive oil) but samples extracted from liver of rats treated with soya bean (G2) showed high degree of DNA fragmentation and this result due to increased in MDA level , the precursor of reactive oxygen species (ROS) and this is in line with **(Lopes, *et al.,* 1998)** as they found that when sperm samples were incubated in the presence of ROS, a significant increase in DNA damage (fragmentation) was detected and addition of antioxidants significantly decreased the amount of DNA damage induced by ROS. Also some isoflavones (soya bean) have been found to be toxic to normal cells in vitro in high doses. They can deteriorate DNA of stem cells, which may lead to leukemia. They've been implicated in increasing the risk of leukemia. Both genistein and daidzein have been shown to accelerate the growth of breast tumors. **Tudisco *et al.,* (2010)** showed also that small DNA fragments can be detected in kid organs when mothers are fed genetically modified soya bean.

Feeding olive oil with soyabean (G4) showed less degree of DNA fragmentation as olive oil prevented the production of oxidative substances produced from soya bean which causes liver cells damage and this result agreed with **Lopez *et al.,* (2004)**, as they reported that the antioxidant activity of extra virgin olive oil as a scavenger of free radical due to its phenolic compounds (hydroxytyrosol and tocopherol) and can minimize the amount of reactive oxygen species (ROS) which mediate cell injury.

C-Histopathology.

Liver: Gross appearance: The liver was congested, enlarged with appearance of greyish yellowish patches on hepatic surface in group (2) and less greyish foci and little congestion in group (4).

Microscopical examination: Liver of rat fed on soya bean (G 2) showed multiple foci of necrotic hepatocytes which replaced by mono nuclear cell infilteration most portal areas were infilterated by mono nuclear inflammatory cells (Fig. 1 and 2) and bile duct proliferation according to **Yoshimitsu *et al.,* (2007).** The marked dilated sinusoids with blood lead to pressure atrophy of some hepatic cells. Activation of Kupffer cells in most hepatic sinusoids could be observed which considered as defense mechanism against toxicity. Some hepatocytes showed binucleated with other hepatocytes had degenerative changes with pyknotic nuclei. The necrobiotic changes of hepatocytes could be explained by the altered foci of mononuclear aggregation as well as near portal areas.

Liver of rat fed on olive oil (G 3) had dilated central vein and hepatic sinusoids engorged with blood. Most hepatic cells were binucleated. Most nuclei were prominent with active nucleolus (Fig. 5). Liver is an interesting organ with high regenerative capacity and complex functions (**Taub, 2004**; **Michalopoulos and Khan, 2005** and **Fausto *et al.,* 2006**) and as the hepatic cells were considered champion cell for regeneration, we detected the more binucleated hepatocytes in group 3 and group 4 which explain the activation of division and regeneration by olive oil.

Liver of rat fed on soya bean and olive oil (G4) had dilated central veins and hepatic sinusoids and engorged with blood as well as activation of Kupffer cells (Fig. 8). Some hepatocytes were atrophied and others had some degenerative changes . Most hepatocytes were binucleated (Fig.7). Some necrotic foci of hepatic tissue could be seen and replaced by mononuclear inflammatory cells (Fig.7 and 9) but the severity of lesion was less than lesion of rats fed on soya only. Fetal exposure to geneistein (phytoestrogen) has been reported to increased mitotic activity **(Foster *et al.,* 2004)**, that explain the increase in the number of mitotic active hepatocytes in group (4). The effects of phytoestrogen exposure on cell proliferation in rats were also concomitant with the effects on

tumor genesis which could be detected by **Kim *et al.*, (2007)** and **Pei *et al.*, (2003).**

Kidneys: Gross appearance: Kidneys showed congestion especially in cortical region with relatively dark red in cut surface in G 2 and less congested in G 4.

Microscopical examination : Kidneys of group 2 showed hyper cellularity of glomeruli with narrowing of Bowman´s space (Fig. 3). Other glomeruli were atrophied with widing of Bowman´s space lining epithelium of renal tubules showed vaccular degeneration in some tubules and coagulative necrosis in others (Fig.4). In some instance mononuclear cell infiltration between renal tubules. Homogenous globular esinophilic structureless substance could be seen in side Bowman´s space as well as tubular lumen .Some renal tubules showed cystic dilatation (Fig.4). The same lesions could be detected in group (4) but less in Fig.11 and 12. Kidneys of group 3 which fed olive oil showed very little necrobiotic changes in renal tubules (Fig.6). Nephropathy in rats which fed on soya bean came in agree with **(Yoshimitsu *et al.*, 2007).** The presence of homogenous esinophilic structure less substance in Bowman´s space and renal tubules were correlated to the deficient in renal filtration which explained the decrease in serum protein. Soya isoflavones have anticancer properties but also possess estrogenic like properties which has raised concern about soya bean consumption among breast cancer **(Reza, *et al.*, 2011).** This substance might be procarcinagenic so that further work is needed to confirm this suggestion.

Conclusion.

In this research , we found that soybean has some harmful features and the difference in our results from the previous researches may be due to differing in quantities of soy used. We found that olive oil enhance the antioxidant synthesis and has beneficial role for general health condition and when added to soy it can ameliorate some its harmful effects.

Table (1). Mean values of oxidative and antioxidive parameter of rats serum fed on soya bean and /or olive oil .

Groups	Conc. /100g ration	MDA (nmol/ml)	GST(U/L)
G1	basal diet	0.654±0.003a	28.86±0.69a
G2	Soya bean 15 g /100g ration	1.26±0.0009b	25.85±0.69b
G3	Olive oil 5g /100g ration	0.590±0.003a	40.12±1.91c
G4	Soya bean 15 g+ Olive oil 5g/ 100g ration	0.629±0.0017a	38.04±2.15c

#Significant at P<0.05 using ANOVA test. a, b. c, Insignificant difference between similar letter using Duncan´s multiple range test at p<0.05 .

Table (2). Mean values of serum cholesterol, HDL, LDL and triglyceride of rats fed on soya bean (15g /100g ration) and olive oil (5ml /100g ration) alone or in combination with each other for 30 days compare ing with control rats. n= 10

Groups	Conc. /100g ration	Cholesterol mg/dl	HDL mg/dl	LDL mg/dl	Triglyceride (nmol/L)
G1	basal diet	90.41±1.97a	24.6±0.796 a	65.9±2.06 a	5.10±0.44 a
G2	Soya bean 15 g /100g ration	92.59±1.14 a	32.16±1.25 c	64.95±1.12 a	5.95±0.39 a
G3	Olive oil 5ml/100g ration	91.55±0.96 a	25.25±0.11ab	65.75±0.11a	4.83±0.45 a
G4	Soya bean 15 g+ Olive oil 5g/ 100g ration	93.37±1.45 a	27.64±2.19 b	61.11±1.11 b	5.73±0.38 a

Significant at P<0.05 using ANOVA test .a, b. c, Insignificant difference between similar letter using Duncan´s multiple range test at p<0.05 .

Table (3). Mean values of some biochemical parameter of serum rats fed on soya bean (15g /100g ration), and olive oil (5g /100g ration) alone or in combination with each other for 30 days comparing with control rats. n= 10 .

Groups	Conc. /100g ration	AST (U/L)	ALT (U/L)	ALP (U/L)	Urea (mg/dl)	Creatinine (mg/dl)	Glucose (mg/dl)
G1	basal diet	49.85±0.17a	36.26±0.15a	201.35±13.83a	14.70±0.23 a	0.781±0.009 a	90.495±0.71 a
G2	Soya bean 15 g /100g ration	60.85±2.67b	41.06±0.70b	264.33±14.19b	24.61±0.33 b	1.14±0.07 b	88.72±2.45 a
G3	Oliveoil 5g/100g ration	49.85±0.15a	35.26±0.15a	201.35±12.83a	13.91±0.499 a	0.698±0.07 a	89.69±1. 5 a
G4	Soya bean 15 g+ Olive oil 5g/100g ration	56.53±2.84b	36.11±2.16a	258.66±13.68c	14.13±0.23 a	0.705±0.06 a	89.66±0.88 a

#Significant at P<0.05 using ANOVA test. a, b. c, Insignificant difference between similar letter using Duncan´s multiple range test at p<0.05 .

Table (4). Mean values of serum protein and its fractions of rats fed on soya bean (15g /100g ration) and olive oil (5g /100g ration) alone or in combination with each other for 30 days comparing with con trol rats. n= 10.

Groups	Conc. /100g ration	T.protein g%	Album. g%	alpha1 g%	alpha2 g%	T.alpha g%	beta 1 g%	beta2 g%	t. beta g%	Gam.1 g%	Gam.2 g%	T.Gam. g%	Globul. g%	AG
G (1)	basal diet	6.89± 0.049 a	2.37± 0.018 a	0.81± 0.015 a	0.35± 0.005a	1.15± 0.019 a	0.53± 0.033 a	0.72± 0.004 a	1.24± 0.007 a	1.82± 0.005 a	0.31±0 .007a	2.13± 0.011 a	4.52± 0.034 a	0.53± 0.003 a
G (2)	15 g /100g ration	7.79± 0.05 b	2.52± 0.01 b	1.01± 0.019 b	.45± 0.007 b	1.46± 0.017b	0.55 0.024 a	0.73± 0.005 a	1.29± 0.022 b	2.09± 0.020 b	0.31± 0.007 a	2.41± 0.027b	5.15± 0.047 b	0.49± 0.005 b
G (3)	5g/ 100g ration	7.18± 0.03 c	2.38± 0.005 a	.87± 0.029 c	.44± 0.012 b	1.31± 0.035 c	0.53± 0.005 a	0.73± 0.006 a	1.26± 0.009ab	1.87± 0.022 d	0.30± 0.010a	2.17± 0.012 a	4.73± 0.026 c	0.50± 0.004 a
G(4)	15g+5g / 100g ration	7.72± 0.03 b	2.62± 0.023 c	.97± 0.017 b	.37± 0.002 c	1.34± 0.013 c	0.48± 0.035 b	0.81± 0.062 b	1.29± 0.065 b	1.95± 0.040 c	0.30±0 .025a	2.15± 0.056 a	4.78± 0.009 b	0.53± 0.015 a

#Significant at P<0.05 using ANOVA test .a,b.c, Insignificant difference between similar letter using Duncan´s multiple range test at p<0.05 .

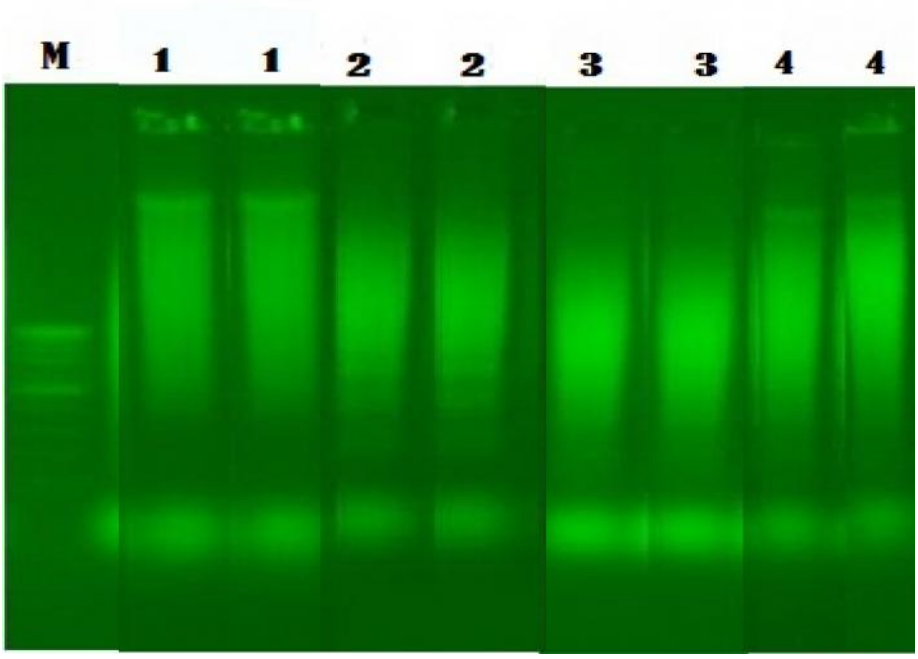

Figure (I) : DNA fragmentation on agarose / ethydium bromide gel. DNA isolated from experimental liver tissues was loaded into 1% (w/v) agarose gels. Marker (!- kb DNA ladder. Lane (1) represents DNA isolated from control rats (G1); lane (2) : DNA isolated from rats fed soya bean (G2); lane 3: DNA isolated from rats fed olive oil (G3) lane 4: DNA isolated from rats fed soya bean and olive oil (G4).

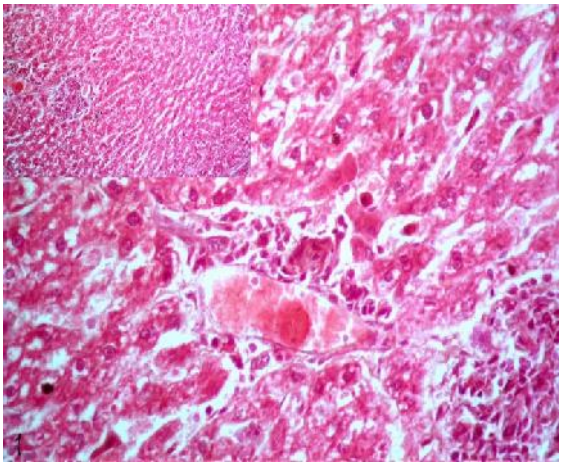

Figure 1. Liver of rat fed on soya bean showed hepatic necrotic foci which replaced by mononuclear cell infilteration as well as dilated central vein and hepatic sinusoids. H&E X400

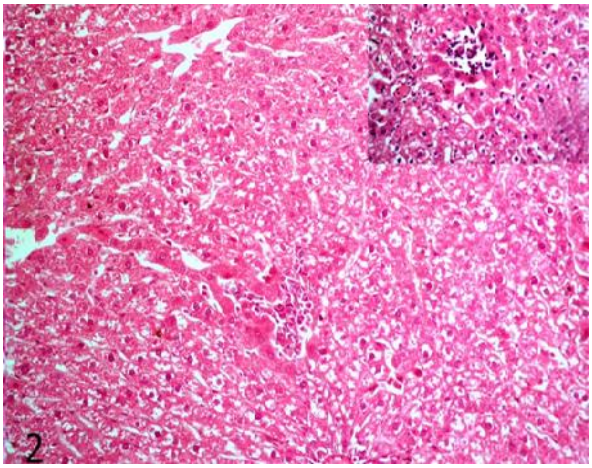

Figure 2: Liver of rat fed on soya bean showed hepatic necrotic foci which replaced by mononuclear cell infilteration. H&E X400.

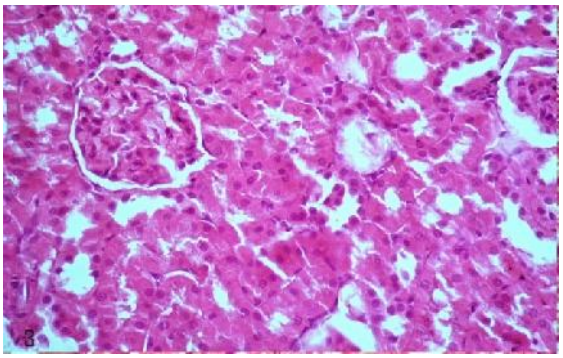

Figure 3. Kidney of rat fed on soya bean showed hypercellualarity of renal glomeruli with narrowing of Bowman"s space as well as necrobiotic changes of renal tubules H&E X400.

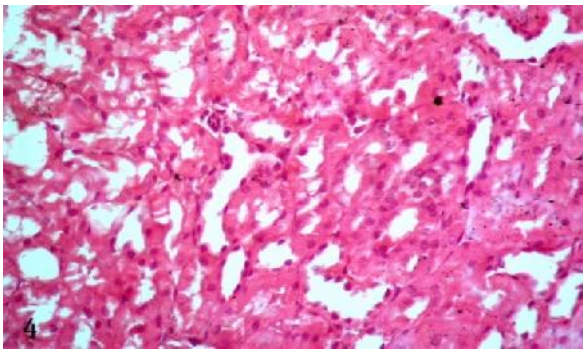

Figure 4: Kidney of rat fed on soya bean showed moderate with cystic dilatation of some renal tubules. H&E X400

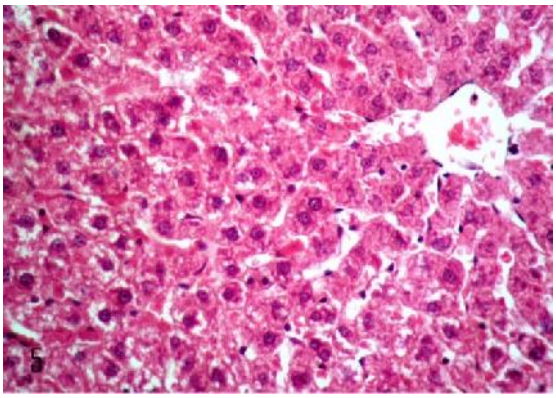

Figure5. Liver of rat fed on olive oil showed slight dilatation of central vein and hepatic sinusoids and more prominent binucleated

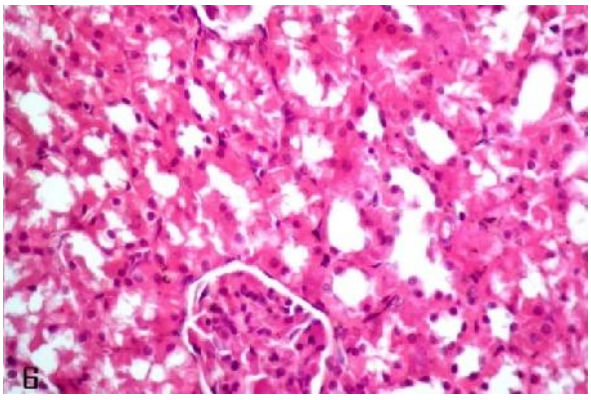

Figure 6: Kidney of rat fed on olive oil showed less degenerative changes in renal tubules. H&E X400 hepatocytes. H&E X400

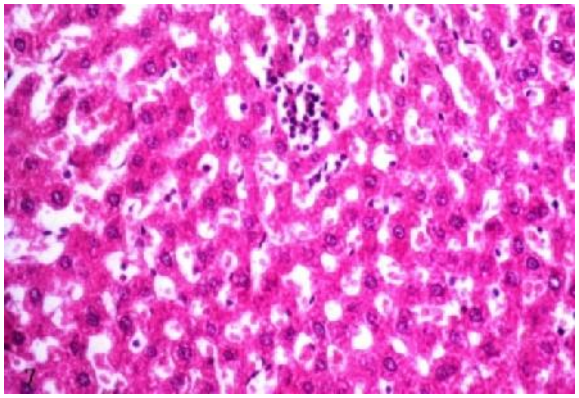

Figure 7. Liver of rat fed on soya bean and olive oil showed less necrotic foci with mononuclear cell infilteration and dilated hepatic sinusoids with prominanet active binucleated hepatocyte H&E X400s.

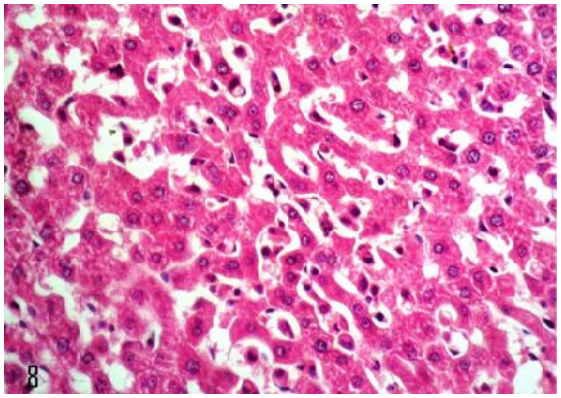

Figure 8. Liver of rat fed on soya bean and olive oil showed less necrotic foci with mononuclear cell infilteration and dilated hepatic sinusoids with prominanet active binucleated hepatocyte H&E X400s.

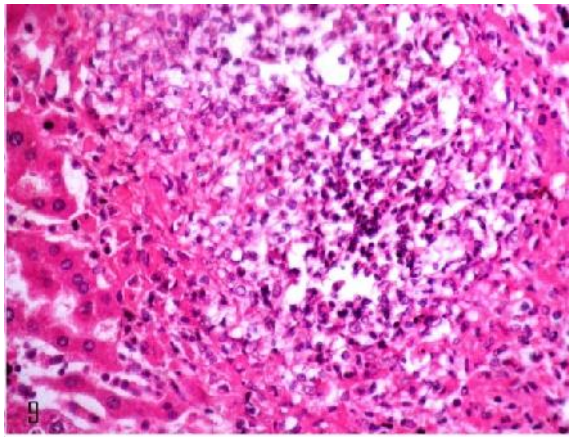

Figure9. Liver of rat fed on soya bean and olive oil showed large healed necrotic focus with mononuclear cell infiltration surrounded by actively dividing binucleated hepatocytes. H&E X400.

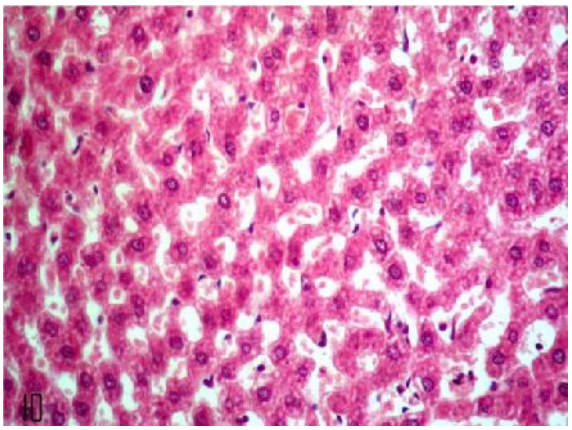

Figure 10. Liver of rat fed on soya bean and olive oil showed more actively dividing binucleated hepatocytes with dilated hepatic sinusoids and activation of kupffer cells. H&E X400

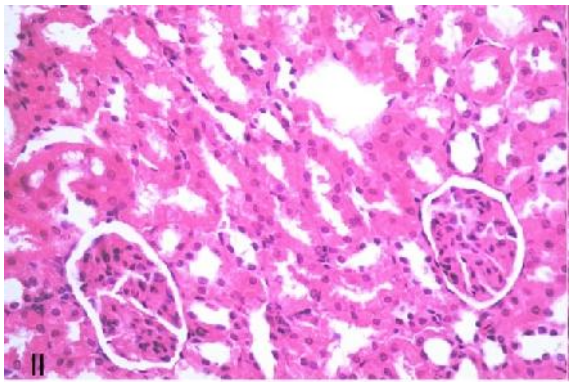

Figure 11. Kidney of rat fed on soya bean and olive oil showed hypercelluarity in glomeruli and less degenerative changes in renal tubules. H&E X400.

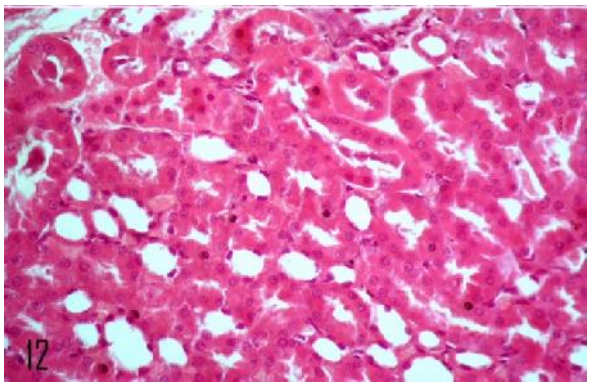

Figure 12. Kidney of rat fed on soya bean and olive oil showed some degenerative changes in renal tubules with homogenous esinophilic structureless material inside some renal tubules. H&E X400.

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