

EVALUATION OF THE PROTECTIVE EFFECT OF JOJOBA NATURAL PRODUCTS ON HEPATOTOXICITY OF DIETHYL NITROSAMINE IN RATS

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Most of the human cancers are consequence of exposure to environmental carcinogens; these comprise both natural and manmade chemicals, radiation, and viruses. Carcinogens could be classified into numerous categories (Reddy *et al.*, 2003). One of these carcinogens is diethyl nitrosamine (DEN) which can be easily synthesized by mixing a secondary amine with nitrous acid under acidic conditions, where nitrous acid is the acid formed when nitrite salts are dissolved in water (Tennant, 1997). Natural products, including plants, are considered as valuable sources for anticancer drug discovery, especially since the advances in understanding the biology of carcinogenesis could help in evaluation of beneficial effects of the natural products in prevention and cure of increasing number of cancers (Schwartzmann *et al.*, 2002). Jojoba

is a perennial woody shrub native to the semi-arid areas of Southern California, southern Arizona and northwestern Mexico. Seeds of jojoba comprise of a mild-gold liquid wax ester that is the main storage lipid within the seeds. Jojoba liquid wax (JLW, conjointly called jojoba oil) comprises of 50% of the seed's dry weight and the maximum amount 97% of it includes a combination of esters of long chain fatty acids and long chain fatty alcohols. A non-saponifiable portion, mostly constituted of phytosterols, has been well described. The JLW exhibits high dielectric persistency, excessive viscosity, high flash and fire point, high oxidative constancy and low volatility. The unique chemical composition and physical properties of JLW makes it a versatile product having many industrial, culinary and medicinal uses (Ranzato *et al.*, 2011). Many

cytotoxic agents and/or DNA damaging agents arrest the cell cycle at the G1, S or G2/M phase and then induce apoptotic cell death (Shapiro, 2001). The study aimed to evaluate the influences of jojoba oil on liver enzyme activities, MDA, histology and DNA content.

MATERIALS AND METHODS

The jojoba oil was obtained from Genetic Engineering and Biotechnology Research Institute, University of Sadat City. The study was held on albino male rats of wister strain, weighing 140-150 gm. Experimental animals were classified into 4 groups (Lee *et al.*, 2007), each group consists of 20 rats:

Group 1: Normal control group, animals of this group were fed the control diet.

Group 2: Animals of this group were injected intra peritoneal by DEN (0.1 ml/100g. b.w twice weekly for 6 weeks).

Group 3: Animals of this group were orally given jojoba oil 2 ml/k. daily for 6 weeks.

Group 4: Animals of this group were orally administrated with jojoba oil 2 ml/k daily for 6 weeks and then injected with DEN (0.1 ml/100g) two times weekly for 6 weeks.

Serum samples were prepared for biochemical analysis. Livers were washed with ice cold PBS and divided in two parts, the 1st part stored at -80°C, and the 2nd part preserved in 10% natural formalin.

Liver enzymes, aspartate transaminase (AST) and alanine transaminase (ALT) were determined in serum according to the method of Reitman and Frankel (1975). The degree of lipid peroxidation in liver homogenate was determined by measuring malondialdehyde (MDA), according to the method of Okhawa *et al.* (1979).

Flowcytometry was used to determine DNA Ploidy by using Dako-Cytometer System. Standardization and initial alignment were performed according to Vindelov *et al.* (1983).

Liver tissues for each rat were preserved in 10% formalin solution and dehydrated in a graded alcohol series. Following xylene treatment, the specimens were embedded in paraffin blocks. Five-micron thick sections were cut and stained with eosin and hematoxylin according to Bancroft and Gamble (2002).

Statistical analysis

The statistical analysis was carried out by one-way ANOVA, followed by t-test. The results were expressed as the mean \pm SD to show variations in a group. Differences are considered significant when ($P < 0.05$).

RESULTS AND DISCUSSION

There were significant difference in serum ALT among the groups ($P < 0.05$). The highest serum ALT level was recorded in the DEN treated group which showed values 314.50 ± 48.96 , while the

lowest levels were in the control group with values 54.57 ± 2.59 (Table 1). The jojoba treated group exhibited value of 65.71 ± 5.87 , which was not significantly different ($P > 0.05$) from the control group (Table 1). The jojoba+DEN treated-group recorded significant decline compared to DEN treated-group, with mean value of 141.66 ± 9.98 .

The mean serum AST levels were significantly changed in the different groups with ($P < 0.05$). The mean serum AST levels in the DEN treated-group were 824.50 ± 88.66 U/L compared to the control group of 176.14 ± 5.84 (Table 1). The mean serum AST level was not significantly different than that of the control group ($P > 0.05$). The serum AST level in the jojoba +DEN treated groups with mean values 358.00 ± 34.53) significantly declined compared to DEN treated group.

The results of antioxidant enzymes and MDA measurement indicated that an enunciate increase of MDA in the DEN treated group; this increase was considerably differed to the other three groups. Furthermore, the raising of MDA was associated with decreasing the level of antioxidant enzymes in the DEN treated group, this reduction was significantly varied in contrast to the other three groups (Table 1). Therefore, the results obviously point out that DEN is affecting the liver negatively by increasing the level of peroxidation and free radical and in the meanwhile decreasing the level of antioxidant protecting enzymes. On the other hand, no significant differences between

the results of other three groups revealed that the jojoba oil treatment has no harmful effect on the liver, and it can effectively maintain the protective enzymatic balance when treated with DEN in rats.

Figure (1) shows the polyploidy levels in 4 groups of rates as determined by flowcytometric analyses. The untreated group showed a single G0/G1 peak with a mean channel number at the (2N) position similar to the reference peak (Fig. 2a). Treatment with jojoba oil did not change polidy level in the liver in all rates showed diploid peaks at the 2N position as observed for the control group. Induction of diethyl-nitrosamine lead to euploidy in several rates as evident by the single G0/G1 peak at the 4N position (Fig. 2c). Pretreatment with Jojoba resulted in a predominate diploid G0/G1 peak at the 2N position with a small G2M peak at the 4N position (Fig. 2d).

Examination of liver of the control animals showed that it is composed of hepatic lobules each of which is consisting of radiating plates, cords or portions of cells forming a network around the central vein (Fig. 2a). Examination of liver sections prepared from rats treated only with jojoba for 6th weeks after treatment had displayed classical hepatic lobules, each was formed of normal strands of hepatocytes radiating from the central vein to the periphery of the lobule (Fig. 2b). In the specimens examined 6 weeks after treatment with DEN, the characteristic cord-like arrangement of the normal liver cells was absent and the normal structural or-

ganization of the hepatic lobules was damaged, in addition, inter hepatic blood vessels portal vein were congested with blood. Some of hepatocytes showed cellular degeneration. Inflammatory leucocytes infiltrations were also observed (Fig. 2c). Examination of liver sections obtained from rats treated with both DEN and jojoba for 6 weeks showed some healthy appearance as the liver tissue displayed a normal architecture, but the portal vein was congested and degenerated bile duct. The hepatocytes, cytoplasm and nuclei showed mostly normal, but blood sinusoids were dilated as shown in (Fig. 2d).

Liver is the primary target site for carcinogen effect of DEN and more than 200 other chemicals comprising pesticides, pharmaceuticals, food additives, and industrial intermediates (Ward *et al.*, 2005).

Following oral, dermal exposure and injection (subcutaneous or intramuscular) of Diethyl-Nitrosamine (DEN) produces carcinomas in several species and DEN induced DNA damage resulting in gene mutations in bacteria and many mammalian cell cultures (Ashby and Patton, 1993).

Many chemotherapeutic agents have originated from natural sources to treat different diseases including cancer. Among these natural sources, Jojoba has proven a good source of such natural products (Ranzato *et al.*, 2011). *Simmondsia chinensis* (link) Schneider belong to the family simmondsiaceae is natives to California. Its seed oil is highly

prized as a medicine for cancer, obesity, kidney disorder, warts, sore heart, and wounds (Wagdy and Taha, 2012).

In the current study, the harmful effect of DEN and the possible alleviating effect of jojoba treatment on the rat liver were investigated. The results showed significant decrease in body weight in DEN treated group, and DEN plus jojoba treated ones compared to the control. These results are in agreement with others who found weight loss in disturbed liver proliferation activity (Zibari *et al.*, 2003).

The liver enzymes ALT and AST in different groups, showed significant increase in DEN treated group when compared to DEN plus jojoba treated one and to the control. No significant difference between the DEN plus jojoba group was found when compared to the control group. The present experimental model using DEN as a hepatotoxicity might explain the recorded of normal level of ALT and AST. The present findings are in agreement with prior report (Zibari *et al.*, 2003).

The results of the current study demonstrated that a pronounced elevation of MDA in the DEN treated group was conveyed with lowering the levels of antioxidant enzyme. These results clearly indicated that DEN was affecting the liver negatively by increasing the level of per oxidation and at the same time decreasing the level of antioxidant protecting enzymes. Moreover, absence of differences between the three other groups confirms that Jojoba oil has no harmful effect on

the liver, and the combined treatment of jojoba oil with DEN can effectively preserve the protective enzymatic balance. The prominent antioxidant activity of jojoba oil was comprehensively investigated in various experimental circumstances, including hepatotoxicity. These results are in agreement with previous studies, where alterations in reduced glutathione and lipid peroxidation (Lu, 1999).

DNA Flowcytometry is a semi-quantitative method for rapid, accurate and quantitative analysis of the DNA ploidy, Proliferative activity and the distribution of cells in the different phases of the cell cycle (Shapiro, 2001).

Flowcytometry is a technique which sorts and analyses cells based on their content of DNA, and can give information about the way where cytotoxic agents affect the cell cycle. To determine if cell growth inhibition involved cell cycle changes, we examined cell cycle phase distribution by flowcytometry (Lee *et al.*, 2003). We have further evaluated the effects of the jojoba oil modes-of-action to the cellular level and we extended our studies on cell cycle effects by flowcytometry. In the current work, all normal control groups, and jojoba treated groups revealed diploid peaks. This finding agrees with the flowcytometric work done by Hsu *et al.* (1999). Similarly all jojoba treated groups with DEN showed diploid peaks. This result is concordant with that reported by Lee *et al.* (2007). While in this study; DEN treated groups

showed aneuploidy, this result agrees with that reported by Brunt and Kraemer (2001).

The ploidy levels in four groups of mice as determined by flowcytometric analyses. The untreated group showed a single G0/G1 peak with a mean channel number at the (2N) position similar to the reference peak. Treatment with Jojoba oil did not change ploidy level in the mice liver all rats showed diploid peaks at the 2N position as observed for the untreated group-U. Induction of carcinogenesis by feeding diethyl-nitrosamine lead aneuploidy in several rats as evident by the single G0/G1 peak at the 4N position. Pre treatment with Jojoba resulted in a predominate diploid G0/G1 peak at the 2N position with a small G2M peak at the 4N position. These results support the hypothesis that jojoba natural products protect liver against carcinogen action. The flow cytometric results exhibited a single diploid peak in the jojoba treated group that was similar to untreated control group, while an additional aneuploid peak was detected in the rats treated with DEN. Furthermore, most of samples in the combined treatment (DEN plus jojoba) revealed a diploid DNA peak without the aneuploid one. The present results are in agreement with other reports that showed the aneuploid pattern in majority of cases bearing adenocarcinoma of the extrahepatic bile ducts (Brunt and Kraemer, 2001; Hsu *et al.*, 1999).

Histological results of jojoba treated group and jojoba with DEN treated

groups showed normal architecture of liver cells and bile ducts similar to that of control group. In DEN treated group there was marked inflammatory of liver cells with mild degenerative changes in the liver of normal tissues. These results are equivalent with the findings of others (Briggs *et al.*, 2009).

Histopathological results jojoba treated group showed normal architecture of liver cells and bile ducts similar to that of untreated group (control. In DEN treated group 42.9% of cases showed remarkable dysplasia of liver cells and inflammatory cells. The current study showed in cases treated with the combination of DEN and jojoba, most of the harmful alterations were apparently rectified. These results are in harmony with the findings of others and the antitumor action was recorded in addition to hepatobiliary cancers, in different other tumors (Ait *et al.*, 2007).

It can be concluded from this study that jojoba oil are a very promising source of bioactive compounds with antioxidant, antimicrobial and anticancer properties. This neglected waste product from an industrial oilseed crop should be given more attention to get optimum benefits for the pharmaceutical industry. The jojoba have a protective effect against hepatotoxicity induced chemically, the mechanism of the protective effect of jojoba may be due to its inhibition of hepatotoxicity bioactivation.

SUMMARY

The current study was designed to achieve the following objectives: Studying

the effect of diethyl nitrosamine (DEN) on rats' liver; and the effect of jojoba oil on the liver enzymes and DNA content. Administration of DEN induced a significant increase in levels of serum ALT and AST. Administration of jojoba oil alone or with DEN caused significant decrease in serum levels of ALT and AST, compared with the control group through the experimental period. The level of lipid per oxidation (MDA) levels increased in DEN treated group. On the other hand animals treated with jojoba and DEN showed a decrease in lipid per oxidation. Compared with the DNA content showed that jojoba oil provoked a massive accumulation of cells in the three cell phases (G0/G1, S and G2/M), this led to the appearance of a diploid DNA content peak in the cell cycle experiments which is characterized to apoptotic cell population. The histological results revealed that jojoba oil improved the alterations caused by DEN.

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Table (1): The level of (ALT, AST and MDA) in the different experimental groups.

Experimental groups	ALT (U/L)	AST (U/L)	MDA (nmol/g protein)
Control	54.57 ± 2.59	176.14 ± 5.84	50 ± 10
Jojoba	65.71 ± 5.87	231.64 ± 11.27	45* ± 5
DEN	314.50 ± 48.96	824.50 ± 88.66	100* ± 5
Jojoba + DEN	141.66 ± 9.98	358.00 ± 34.53	70 ± 10

J: Jojba

DEN: diethyl-Nitrosamine

Data are represented as mean ± SE. Significant compared to control.

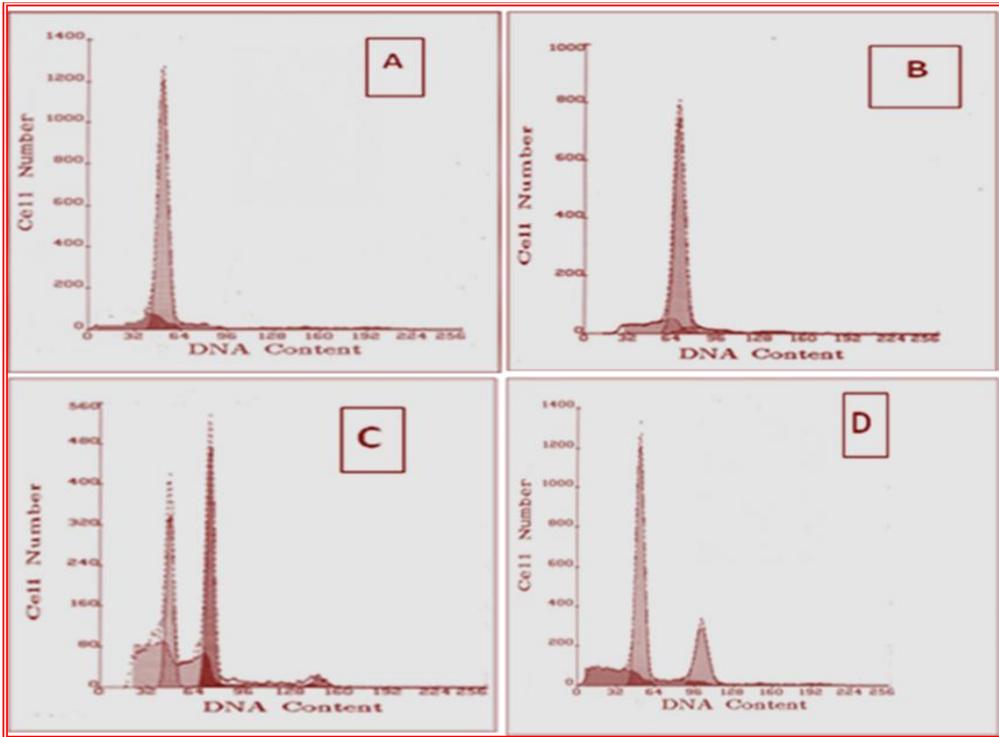


Fig. (1): Flowcytometric analysis (A) Control group (one diploid peak); (B) jojoba treated group (one diploid peak); (C) DEN treated group (aneuploid peak pattern) and (D) DEN + jojoba treated group.

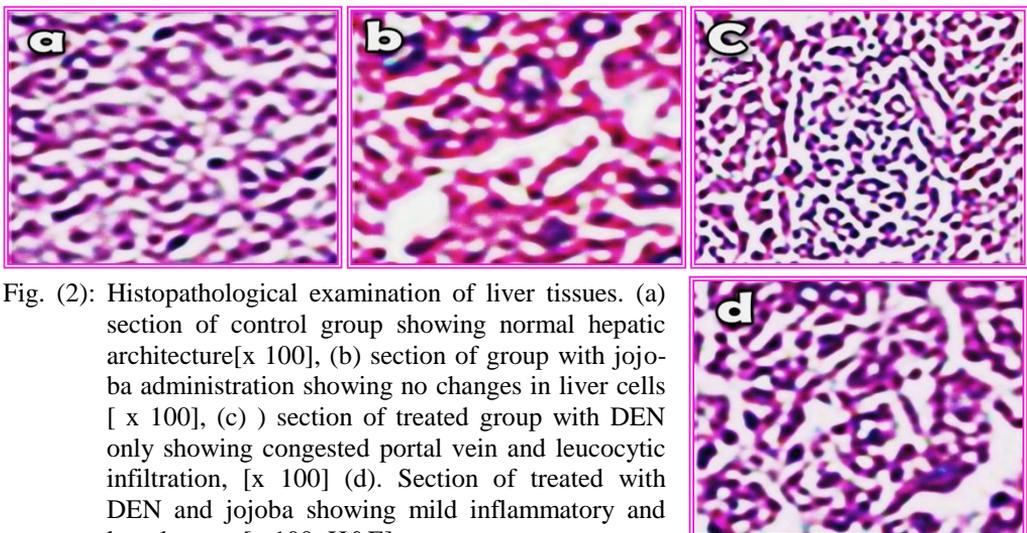


Fig. (2): Histopathological examination of liver tissues. (a) section of control group showing normal hepatic architecture [x 100], (b) section of group with jojoba administration showing no changes in liver cells [x 100], (c) section of treated group with DEN only showing congested portal vein and leucocytic infiltration, [x 100] (d). Section of treated with DEN and jojoba showing mild inflammatory and lymphocytes [x 100; H&E].