Anticancer and antioxidant effects ofparsley (*Petroselinum sativum*) andSpinach (*Spinocia oleracea*)seed oils against chemically induced liver cancer in rats

**ABSTRACT**

Parsley (*Petroselinum sativum*) and spinach (*Spinocia oleracea*) seeds were used for production of seed oils used in the present study. Antioxidant and anticancer activities of Parsley (*Petroselinum sativum*) and spinach (*Spinocia oleracea*) seed oils (PSO and SSO respectively) against diethylnitrosamin (DENA)-induced liver carcinogenesis in male albino rats were investigated. Administration of DENA to rats showed significant increased in alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in sera of rats. Significant decreases in plasma and tissues antioxidants as glutathione transferase (GSH-T), glutathione peroxidase (GSH-P), glutathione reductase (GSH-R) and superoxide dismutase (SOD) activities were observed in DENA‑induced rat group. Administration of parsley (PSO) and spinach (SSO) seed oils with DENA-induced rats, exhibited improving in biochemical changes of liver function enzymes. A marked reduction were observed in the levels of ALP, ALT and γ-GT in sera of rat groups given PSO and SSO seed oils as compared to DENA control rats groups, indicating protective effects of both seed oils against harmfull and toxicity of DENA. Significant decreases in the levels of lipid peroxidase (LP) in sera of rats administered PSO and SSO seed oils compared to those of DENA control rats. Higher significant decrease in the level of LP was observed in sera of rats administered PSO seed oil more than those given [SSO](https://en.wikipedia.org/wiki/Pumpkin_seed_oil) seed oil. PSO and SSO seed oils showed more effective for inhibiting DENA-induced liver cancerthrough evaluation and determination of tumor markers(CEA, CA19-9, CA15-3and CA125) in sera of DENA-induced liver carcinogenic rats groups compared versus carcinogenic control rat group.The present results showed the activity of antioxidant enzymes (GSH-T, GSH-P, GSH-R and SOD) were increased significantly in liver, kidney and heart of rat groups treated with PSO and SSO seed oils as compared to those of DENA control rats.The most significant findings of the present study are the PSO and SSO seed oils have shown beneficial effect not only on liver cancer but also on antioxidant defense enzyme activities in DENA- induced [liver carcinogenesis in rats](http://www.scialert.net/asci/result.php?searchin=Keywords&cat=&ascicat=ALL&Submit=Search&keyword=diabetic+rats) as well as protect cell against DENA oxidative stress by antagonizing DENA toxicitys. These finding suggested that the seed oils (200 mg/kg b.w.) could be apotential compounds used in protective and treatments of liver tumors. According to these observations, the use of PSO and SSO seed oils can be recommended as antioxidant and anticancer agents for production of many types of inexpensive seed oils have shown beneficial effects in treatments and combating oxidative damages of liver carcinogenesis.The most findings of the present study are the possibility of produced many types of inexpensive seed oils, have shown beneficial effects on chemically induced liver cancerin rats.indicates both seeds could be used as food, food purposes, pharmaceutical and drugs for treatment of different diseases. Supplemented diets with parsley and spinach seeds containing antioxidant and treatment agents effectively used in the chemotherapeutic treatment of different cáncer types particularly liver cáncer. Thus, oils can be used as strong sources of novel anticancer and antioxidant drugs for cancer treatments.

**KEY WORDS**: Parsley, Spinach, Seed, Anticancer, Antioxidant, Rat.

**1. INTRODUCTION**

Cancer is the second leading cause of death in economically developed countries and the leading cause of death in developing countries due to the lack of diagnostic techniques, standard methods and higher cost of the treatments [1,2]. Developing countries have more number of cancer incidence cases compared to the developed countries [3,4]. Liver the abdominal largest organ have effective roles in metabolism, detoxification and excretion of toxins The most drugs ingested orally pass through the liver and metabolized into intermediates toxic with adverse side effects represented the liver injuries [5,6]. Many investigators [7, 8] reported the liver cancer represents a great public health problem between six most common cancers in the world and represents the third leading cause of cancer mortality worldwide in the last decades [2,4]. Liver cancer caused by oxidative stress and inflammation cause cancer-related mortalities [3, 9,10,11,12], reported the liver cancer was arises in the setting of chronic liver disease. Chemotherapy and radiotherapy currently used in cancer treatment were more expensive and have induced drug resistance that cause toxic to healthy cells leading to some side effects [13,12]. Drug resistance, side effects and higher cost of the current therapeutic leads to the scientests search about new compounds or drugs from natural inexpensive sources for cancer treatment [14,15,16]. Continuing need for effective inexpensive anticancer agents, several researchs are concentrates for production of natural bioactive compounds used in treatment of different type of cancers [17, 18,19]. Other studies produced novel anticancer drugs from natural products with potential antitumour and chemopreventive activities [14,15, 20]. Plants have been common among people were found extensively utilized in food, pharmaceutical and medical industries to synthesize and produce drugs have biological activities in treatment of most diseases [15,16, 21]. Many investigators [6,22,23,24] reported the plant extracts were used as apotential treatment for cancers and induce apoptotic cell death of human colon and hepatoma cells in vitro and in vivo. Different compounds produce from pland origin like polysaccharides, alkaloids, saponins, triterpenes, polyphenols and flavonoids have shown antioxidant and anticancer properties in vitro and in vivo [24,25]. Plant drived compounds have wide applications in cancer therapeutics due to lower cost and induce lesser side effects compared to synthetic drugs [14,26,27]. Different anticancer drugs such as paclitaxel, vinblastine, vincristine and colchicine are plant derived compounds have been aproved effectively anticancer drugs used in clinical practice against some cancer types [14,28]. Different deived compounds from plant origin are used in structure of anticancer drugs development [15,27], they reported the anticancer drugs from natural products have low cost and exhibited several effective actions of chemotherapy against resistant cancer cells. Many studies suggested certain natural product produced from plant sources might be useful as anticancer and chemopreventive agents in a variety of bioassay systems and animal models due to phytochemical constituents [18, 20,27,29]. Several investigators [11,30, 31,32], stated the diets, nutrition and oxidative stress exhibited reduction of antioxidant defenses against cancer cells that consider the main factor in human carcinogenesis and chronic diseases development resulting effects of most cancer types Other investigators [26,32] indicated the diets included high fruits and vegetables containing some phytochemicals provide cancer chemoprevention and reduce the risk in developing of chronic diseases including cancer by interfering with cell cycle inducing apoptosis [21,33, 34]. Phytochemicals exhibit antitumor activities through improvement the defences of antioxidant enzymes, remove oxidative stress, followed inhibition of cacinogenesis and direct absorb the reactive oxygen species [24,34,35]. Phenolic compounds and polyunsaturated fatty acids, the major phytochemicals, are widely distributed in fruits and vegetables [35,36,37], may contribute to health-promoting effects through powerful antioxidant properties, decrease metastasis, induce apoptosis, and inhibit cell proliferation [31,38]. Anticancer drugs used in medicine are produced from fruits and vegetables involving phenolic, flavonoides and polyphenols as different kinds of antioxidants are scavengers of free radicals [16,30] and are modulated during carcinogenesis or after tumor formation [25]. Antioxidant substances such as terpenoids, phenolic, flavonoids and lignans of plant sources were discovered as natural compounds have been capable of scavenging free superoxide radicals, protecting biological system against harmful effects of oxidative processes and play an important role in cancer treatment [37,39,40]. Natural compounds with antioxidant activity can target tumor cells after disease occurrence, directly inhibit cell proliferation and prevent tumor recurrence or metastasis [22,25,41]. Several studies [4,19,37,42] stated the natural antioxidant compounds have anti-inflammatory, antitumor and anticarcinogenic activities. Some studies [31, 43] established the anticancer effect of antioxidants as inhibit cancer cell proliferation, differentiation, induce apoptosis, metastasis and interfere in angiogenesis [37,38]. Free radical or reactive oxygen species is the main factor in the lipid peroxidation formation, consequently damage the cell membrane resulting from toxicity leads to hepatic dysfunction and reduced the glutathione responsible for removing free radicals [11,44, 45]. Plant seeds as natural source, considered as a part of human culture used by ancient peoples due to its contents of various chemical materials or compounds used as food, feed or in medicine [31, 32, 36,42]. Plant seeds have rich nutritional and nutraceutical ingradients used for treatment and protection against most diseases [4, 21, 46,47]. The evidence of the previous studies [10,23,37] revealed the consumption of plant seed results in treated and protection against chemically induced colon cancer [16]. Seed extracts showed anticancer and pharmacological effects in vitro, in vivo and in medical trials [15,29,40]. Oils extracted from seeds of different plants were found to have nutritional quality used as edible oil food ingredients in various food ietms and consumed in appreciable amounts in most diets [32,48, 49]. [Oils](https://en.wikipedia.org/wiki/Vegetable_oil) are biological mixtures include glycerol and chain of fatty acids [7,38,50] reported the oils contain fatty acids referred to as prebiotics, improving the health state of humans and may be partially responsible for their physiological effects. Oils consider one of plant-derived compounds, were found to be used in treatment of diabetes [51], cardiovascular and other various diseases [39,52]. Oils extracted from plant seeds have antimicrobial, antifungal and antitumor [36,42,43].Seed oils are non-toxic and biodegradable that consequently suitable for different pharmaceutical and biomedical uses which play important roles in several physiological and pathological conditions [19,21,32,36]. Plant seed oils can be considered as bioactive molecules in medicine have been demonstrated to have antitumor [43] and chemopreventive effects [4,18,26]. Other investigators {42,53] reported the seed oils were found to be used as antiviral, antibacterial, anticancer and antioxidant agents [16,25,36].Seed oils of different plants have been shown the potential health impacts in preventing some diseases including cancer and have antiinflammatory [41], antiproliferative [22, 25], anti-angiogenic [43], antigenotoxic [37], antimicrobial [16] and anticancer activities [17,18] when they were used certain seed oils in cancer therapy against tumors development. Higher anticancer ingredients, including fatty acids, phenolic and flavonoid as antioxidant compounds being associated with improved human health were found in seed oils [7,18,25,38] Seed oils with their constituents of fatty acids and other phytochemicals posses various bioactivities including cytotoxicity [36,54], anticancer [10,35]antidiabetic [16,51]. Seed oils were found to be used in treated and protection against chemically induced colon and hepatocellular carcinoma using rats (10,23,,55]. Other studies [29,35] reported some plant seed oils containing phytochemicals and antioxidant compounds were beneficial to protect the mucosa against chemical carcinogénesis and protect the liver against lipid peroxidation impairment in antioxidant status induced by CCl4 [7,10,16,56]. However, the addition of synthetic antioxidants to the oils and foods were considered one of the most efficient ways of lipid peroxidation inhibition have some undesirable side effects [38,57,58]. Oil extracts, have become interesting as an alternative to synthetic antioxidant agents in food, alternative medicine, natural therapy and pharmaceutical industries [56,58]. In recent years, there is powerful in studies on new natural healthy antioxidants replace synthetic antioxidant compounds in food and pharmaceutical industries [20,59,60]. Natural compounds with antioxidant activity can directly inhibit cell proliferation and stimulate the immune system [54,59].Various studies [61, 62, 63] indicated the natural antioxidant compounds have able to delay or inhibit the oxidation of lipids resulting prevent the damage of cells caused by reactive oxygen. The primary role of antioxidants is to prevent oxidative lipid damage produced in proteins and nucleic acids by reactive oxygen species, including reactive free radicals [30,62,64]. Antioxidant properties are involved in protection of the liver tissue against hepatotoxin-induced toxicity (6,7,44]. Parsley (*Petroselinum sativum*) and Spinach (*Spinocia oleracea*) seeds are commonly used as food or in medicine and consider a good sources of diet health-promoting ingradients contains fatty acids, phenolic and flavonoid compounds have various biological properties [23,49,59,64]. However, there is a littele research on Parsley and Spinach seed oils antioxidant or anticancer on chemically induced hepatocellular carcinogenesis in vivo. Therefore, the present study was done to investigate the antioxidant and anticancer activities of the produced parsley and spinach seed oils (PSO and SSO) against DENA-induced liver cancer using male albino rats.

**2. MATERIALS AND METHODS**

**2.1. Materials**

**2.1.1. Seed samples**

Seed samples of Parsley (*Petroselinum sativum*) and Spinach (*Spinocia oleracea*) were obtained locally from markets in Egypt and damaged seeds were removed, washed with tap-water followed by distilled water and drying in an oven at 50 C for 48 hours according to the Association of Official Analytical Chemists [65]. Seeds were then ground using food grinder (mincer) to a very fine powder, sifted through a 16mesh sieve, packed in bags, and stored at room temperature till used.

**2.1.2. Carcinogenic material**

Diethylnitrosamine (DENA), fatty acid standards and all other chemicals used in the present study were purchased from Sigma-Aldrich® chemie, Gmbh, Riedstr. 2, D-89555 Steinheim, Germany.

**2.1.3. Animals**

Thirty five male albino rats, 10 weeks of age, weighing about 180±1.4g were purchased from the National Research Center for biological products. The rats were randomly divided into five groups (7rats/group) were housed in a wire screen cage. The rats had free access to fed commercial diets and tap water. The animal room was controlled (25±1ºC) and had a 12-hour light-dark cycle and humidity at 60±5%. The rats were acclimatized for a period of two week before the experiments began.Three groups of rats were administrated for 6 weeks (five/week) intraperitoneal injections of diethylnitrosamine (DENA) at a dose of 20 mg/kg body weight [10,55]. One rat group administrated DENAwas maintained without any treatment over experimental period (20weeks) and used as liver carcinogenic control rat group (C). Other two rat groups from3 groups of rats administrated DENA for 6 weeks (five/week) were then treated with daily oral doses (200mg/kg body weight) of parsley (PSO) and spinach[(SSO)](https://en.wikipedia.org/wiki/Pumpkin_seed_oil) seed oils (C/PSO group and C/SSO group respectively) from week 7 till the end of experimental period (20weeks).Remaining two groups of rat were administrated daily with oral doses (200 mg/kg body wt) of PSO and SSO seed oils for 6 weeks from the first week and then they were administrated for 6 weeks (five /week) intraperitoneal injections of DENA at a dose of 20 mg/kg body weight and treated with daily oral dose (200mg/kg body weight) of PSO and SSOseed oils (PSO/C group and SSO/Cgroup respectively) from week 7 till the end of experimental period (20weeks).The experimental protocol was done according to the method as previous described [66].

**2.2. Methods**

**2.2.1. Sample preparation**

Seeds Parsley (*Petroselinum sativum*) and Spinach (*Spinocia oleracea*) of using cold-pressed extraction process at low temperature [67]. The obtained seed oils of Parsley (PSO) and Spinach (sSO) were kept in dark bottles and stored at -18°C till used.

**2.2.2. Chemical analysis**

Total carbohydrate was determined in seeds using phenol-sulfuric acid method [68].Protein content was determined [69]. Total lipid was separated and estimated [70,71]. Total phenolic content of each seeds was determined by using the Folin-Ciocalteu reagent [72]. Colorimetric aluminum chloride method was used for flavonoid determination [73]. Phenolic was expressed as mg of Gallic Acid Equivlents (GAE). Flavonoid was calculated as mg Catechin Equivalents (mg CE). All chemical analyses were carried out in triplicate and the mean values were calculated.

**2.2.3. Oils extraction**

Oils were extracted three times from Parsley (*Petroselinum sativum*) and Spinach (*Spinocia oleracea*) seed separately using cold-pressed extraction process at low temperature [67], the seed oil samples obtained from Parsley (PSO) and Spinach (SSO) were kept in dark bottles and stored at -18°C till used for the analysis.

**2.2.4. Fatty acids analysis**

PSO and SSO seed oil samples obtained were used for fatty acids analysis by gas liquid chromatography [74]. Fatty acid methyl esters of the all oil samples were prepared [75], and subjected to Gas liquid chromatography (GLC) for [fatty acid](http://www.scialert.net/asci/result.php?searchin=Keywords&cat=&ascicat=ALL&Submit=Search&keyword=fatty+acid)s analysis. The fatty acid composition of seed oil samples was identified by GLC [76].

**2.2.5.Cytotoxicity**

Cytotoxicity test of seed oils (PSO and SSO) were measurement [77,78], PSO and SSO were administered orally to overnight fasted rat at the doses of 100, 200 and 300 mg/kg body weight (b. w.). After administration PSO and SSO seed oils, the rats were observed continuously for 72 hours, following their general behavior, toxicity, physiologically reaction and mortality [79 ,80].

**2.2.6. Cancer induction**

## Induction of liver (hepatocellular carcinoma) experimentally in rats was done using diethylnitrose amin (DENA) according to method described by other investigators[10,16, 55].

**2.2.7. Samples preparation**

At the end of experimental period (20weeks), blood samples were drawn from 7 rats per each group separately using capillary tubes, centrifuged at 4000xg for 10 min. Separated sera or plasma were stored at - 60ºC till used. Liver, kidney and Heart tissues were removed immediately, weighed, washed (using saline 0.9%), minced and homogenized (10% w/v) separately with cold sodium potassium phosphate buffer (0.01M, pH 7.4) using homogenizer (Mechanika precyzyjna warszawa model MPW-309, Poland). The homogenates were centrifuged at 15,000g for 20 min at 4ºC and the resultant supernatants were stored at - 70ºC and used. for estimation of the activities of glutathione-s-transferase (GSH-T), glutathione peroxidase (GSH-P), glutathione reductase (GSH-R), superoxide dismutase (SOD) and other biochemical parameters.

**2.2.8. Biochemical parameters**

Alkaline phosphatase (ALP) level was carried out [81]. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured [82], using kits of QCA, Spain. Gamma glutamyl transferase (γ-GT) was carried out according to the kinetic colorimetric method [83], using Biodignostic kits, Egypt.Total protein was also estimated [84] using Biodignostic kits, Egypt. Serum albumin level was measured [85]. Globulin was calculated by subtracting albumin form the total protein [86]. GSH-T (EC 2.5.1.18) and GSH-P (EC1.11.1.9) activities in plasma and homogenates of liver, kidney, and heart tissues were assessed [87,88].GSH-R (EC1.6.4.2) activity was assayed using the method of Goldberg and Spooner [89]. SOD (EC 1.15.1.1) activity was measured as described byElstner et al.[90]. Lipid peroxidase (LP) was also estimated [90,91].Determination of carcinoembryonic antigen (CEA) was performed with commercially available Enzyme Immunoassay Kit (Bio Check, Inc. catalog number: BC-1011) according to the method of Uotila et al. [92]. Carbohydrate antigens (CA 19-9 and CA 15-3) and cancer antigen 125 (CA 125) were performed with commercially available Enzyme Immunoassay Kit [93].

**2.2.9.Statistical Analysis**

Data from the biochemical analysis was statistically analyzed using student T-test [94[.

**3. RESULTS AND DISSCUTION**

**3.1. Chemical composition of Parsley (*Petroselinum sativum*) and Spinach (*Spinocia oleracea*) seeds.**

Two seed samples of parsley (*Petroselinum sativum*) and spinach (*Spinocia oleracea*) seeds were obtained from local markets. Results in Table (1) show different contents of protein, lipid, carbohydrate, phenolic, flavonoid and ash were estimated in Parsley (*Petroselinum sativum*) and Spinach (*Spinocia oleracea*) seeds powder used in the present study. Parsley seed contains higher percentages of protein and lipid (28.20 and 14.80% respectively) than those of spinach seeds (24.60 and 12.60 respectively). Data in Table (1) showed spinach seed contains high content carbohydrate (58.40%) than that of parsley seed (54.40%). Results of the present study showed the seed of spinach contain large amounts of phenol and flavonoid (42.20% and 24.20% respectively) than that of parsley seed (36.80 % and 22.20% respectively). These results are in accordance with those obtained by other investigators [95,96,97] they found higher protein content in different plant seeds. Lower levels of protein were found in Lettuce ,radish and coriander (10.2%) seeds [98,99,100]. Other investigators found the protein contents of some plant seeds was 21.70% [101,102,103].

Table 1. Chemical composition of different plant seeds.

|  |  |  |
| --- | --- | --- |
| Ingradients | Parsley (*Petroselinum sativum*) | Spinach (*Spinocia oleracea*) |
| Protein (%) | 28.20 | 24.60 |
| Lipids (%) | 14.80 | 12.60 |
| Cabohydrate (%) | 54.40 | 58.40 |
| Ash (%) | 2.60 | 3.40 |
| Phenolic (mg GAE/g DW) | 36.80 | 42.20 |
| Flavonoid (mg CE /gDW) | 22.20 | 24.20 |
| Oil yield | 16.02 | 14.98 |
| SFA (%) | 8.20 | 10.80 |
| MUFA (%) | 57.40 | 59.00 |
| PUFA (%) | 34.40 | 30.20 |
| P/S ratio | 4.20 | 2.80 |

Mean values of three samples

Results also showed the lipid contents were higher in sesds of parsley and spinach (14.80% and 12.60% respectively) as shown in Table (1) These results are in the range with those reported by several investigators [36,98,99,100]. Highest levels of carbohydrate were observed in spinach (58.40%) more than that of parsley (54.40%). Similar results were obtained by several investigators [101,102,103] they were used different seeds of radish (50.2%), dill (58.6%), chickpeas (52.8%) and lettuce (52.4%). Other investigators [67,97] obtained low carbohydrate contents in seeds of [purslane](file:///C:\wiki\Portulaca) (48.2%) and rapeseed (30.6%). The differences in the composition of both seed samples were quite close to the previous finding [38,104]. Results concerning phenolic and flavonoid contents of parsley and spinach seed samples are also presented in Table (1). The higher phenolic and flavonoids contents were recorded in spinach (42.20% and 22.20% respectively) more than that of parsley (36.80% and 24.20 % respectively). These results are higher than those reported by other studies [59,96,97], they reported the total phenolic content was ranged from 12.7 to 25.6mg/100g in some plant seeds. Other investigators [29,67,105], reported the phenolic content was varied from 15.9mg/g to 22.7mg/g in different plant seeds which are lower than our results obtained of parsley and spinach seeds samples. Lower levels of phenolic contents (2.8 - 4.4 mg/g) were obtained by other investigators used [purslane](file:///C:\wiki\Portulaca), radish and dill seeds [36,99,103]. Flavonoid showed higher content in parsley and spinach seed samples (22.20% and 24.20% respectively) than those reported by other studies [99, 106] found the phenolic contents ranged from 0.98 to 3.35mg gallic acid equivalents per gram seeds. Flavonoid contents were obtained from three samples of *Eurca sativa* seed ranged from 23% to 25% [96]. Other studies [29,98,100] showed the lower flavonoid contents (0.2-4.2%) in coriander, chickpeas, radish, [purslane](file:///C:\wiki\Portulaca) and Lettuce seeds [60,101,102].Therefore, both seeds used in the present study are recognized as dietary elements with important effects on human health and could be used as food, food purposes, pharmaceutical and drug for treatment of different diseases.

**3.2. Oil yield and fatty acid contents**

Oil yield of the parsley and spinach seeds were obtained and recorded in Table (1). Parsley and spinach seeds were yielded the maximum oil contents (16.02% and 14.98% respectively), Variability in oil contents between parsley and spinach seed oils (PSO and SSO) revealed their potential and used both seeds as natural sources for higher seed oils production. Oils were extracted from parsley and spinach seeds using cold-pressed methods [60,67] demonstrates the method feasibility to obtain different types of seed oil suitable for food application through 2h.in laboratory. The present results are in agreement wth those reported by other investigators [64,97,106] using cold-pressed extract for production of safflower, pumpkin, black caraway and hemp seed oils. The finding of slightly reduction was observed in rapeseed cold-pressed oil yield compared to Soxhlet extraction using different solvents [19, 95,106]. Results presented in Table (1).showed the presence of maximum oil yields obtained from Parsley seeds (16.02%) was higher than that of spinach seeds and 14.98%). These results are in the range with those reported by other investigators [98,100], reported the lower oil yield was present in purslane, lettuce and radish seeds (14.20 %-18.20%). Other studies [4,18,36,102,103] found lower oil yield content in coriander, chickpeas and dill seeds (0.31% -8.50%). Seed oils obtained from parsley seed (PSO) and spinach seed (SSO) in the present study were used for analyses and determination of fatty acids composition using Gas Liquid Chromatographic. Generally, seed oils have saturated and unsaturated fatty acids as major contents (Table 1). The percentages of saturated fatty acids (SFAs), monounsaturated fatty acids (MUSFA), polyunsaturated fatty acids (PUSFAs) and the values of PUSFAs /SFAs ratio (P/S) were determined in the obtained seed oil samples (PSO and SSO) as shown in (Table 1). Results showed higher level of PUSFAs in PSO (34.40%) and SSO (30.20%) than that of SFAs (8.20% and 10.80% respectively). USFAs to SFAs varied from 4.20 for PSO to 2.80 for SSO, showing that there was higher extraction of polyunsaturated compounds than that of saturated compounds [36,50,96,98], indicated the various seed oils have different values of USFAs to SFAs. The relationship between PUSFAs and SFAs content is expressed as P/S ratio (Table 1). This value is an important parameter for determination of nutritional values of these oils. The higher value for P/S ratio was found for PSO (4.20) but low P/S ratio was found in SSO (2.80). Furthermore, the P/S ratio obtained was ranging from 2.80 to 4.20, showing the predominance of PUSFAs to SFAs in the present seed oil samples (SSO and PSO respectively). Oils with higher value of P/S ratio than 1.0 are considered to pocess varios nutritional values. Furthermore, P/S ratio obtained was 2.80 and 4.20 in SSO and PSO respectively, indicated the cold press extraction exhibited higher extraction of PUSFAs constituents than that of SFAs showed the predominance of polyunsaturated to saturated fatty acids in POS and SSO seed oil samples used in the present study.These results are in agreement with those obtained by other investigators [36,74,107] found the PUSFAs were predominants than that of SFAs constituents of different oil extracts. Several studies indicate that higher value of P/S ratio means a smaller deposition of lipids in the body [50, 104,108] reported higher value for P/S ratio was found for safflower oil (10.55) and the lowest for palm kernel oil (0.016). These results are in line with the data obtained from the literature [50,109] reported the Canola oil and linseed oil differed from the others by presenting the levels of PUSFAs for canola oil more than that of linseed oil. However, PUSFAs, particularly ω-3 (C18:3) plays an important role in the regulation of biological functions and oxidation reaction of different oils. The composition of USFAs and SFAs were tested in the obtained PSO and SSO seed oil samples. Analyses of PSO and SSO seed oil samples revealed there were different variations in their contents of SAFAs and USFAs, between both seed oil samples (Figure I). Results in Figure (1).indicated the presence of 7 main fatty acids (C18:3, C18:2, C18:1, C16:0, C18:0, C20:0 and C22:0) were detected in PSO and SSO seed oil samples under the present study. These results in consistency could be attributed to growing condition and method of extraction [36,100]. Other studies [98,110] detected 5 main fatty acids in purslane and lettuce seed oils. Results in figure (I) showed the presence of different levels of oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) in both obtained seed oils (PSO and SSO). Results showed higher percentage of PUSFAs, were linolenic acid (C18:3) in PSO (12.20%) but lower level of linolenic acid (C18:3) was found in SSO, (10.20%). These results were quite close to the finding of other investigators [18,98,100], found C18:3 content was 10.56% in some seed oil samples. The content of C18:3 ([ω-3](https://en.wikipedia.org/wiki/Omega-6_fatty_acid)) in PSO and SSO seed oil samples (12.20% and 10.20%respectively) were higher than those reported by other investigators [38,111], they reported the maximum C18:3 content in pumpkin seed oil was 8.84%. Other investigators [95,102,103] found the C18:3 contents in rapeseed and coriander seed oils were ranged from 5.20% to 15.0%. The analyses of PSO and SSO (Figure 1) showed the higher linoleic acid (C18:2) contents (22.20% and 20.60% respectively). These results are higher than those repoted by other workers [29,102,103,112,113] using coriander, watercress and lettuce seed oils (12.3%- 16.6%).. Highest level of oleic acid (C18:1) was observed in the obtained PSO and SSO seed oil samples (57.40% and 59.00%) as shown in Figure (1). These results are quite close in agreement to the previous finding (49,50,95], they found the C18:1 content was varied from 57.85% to 64.63% in *B. napus* seed oil. However, the content of C18:1 was 22.2% in chickpeas seed oils [101] while 20.9% of C18:1 was obtained from radish seed oils production [23, 98]. The percentage of 7.5-15% C18:1 was found in seed oils of coriander , and watercress [53,60,102,103]. Results showed higher percentages of SFAs in PSO and SSO seed oil samples (8.20% and10.80% respectively), as shown in Table (1). Similar results of SFA content (6.9% - 9.2%) were obtained [36,38,100] when used different seeds for oils production. However, the present results are lower than those reported by other investigators [53,102,103], found 24.01% and 22.4% of SFAs content in oils produced from pumpkin and safflower seeds respectively. Palmitic acid (C16:0), stearic acid (C18::0), arachidic acid (C20:0) and behenic acid (C22::0) were identified in PSO and SSO seed oil samples (Figure 1). These findings are in line with the literature data [25,50,101] used different seed oils. C16:0 and C18:0.are the major SFAs contents for PSO (4.80% and 2.00 % respectively) and SSO (6.60% and 2.80% respectively) seed oil smples. These results are in the range with those finding by other investigators [49,95,99,104], found C16:0 content was 5.7% while the C18:0 content was ranged from 3.0% to 5.0% in safflower and rapseed seed oils.. The present results of C 16:0 and C18:0 in PSO and SSO seed oils are lower than those obtained by many investigatord [53,102,103], stated differnent variations were found in the content of C16:0 (20%–22%). Results showed C20:0 percentages were similar (0.80%) in PSO and SSO seed oils (Figure 1). The present results of C20:0 percentages (Figure 1) are consistant with the previous finding [29,97,100,101], reported different variations (0.20-1.40% ) were observed in the conent of C20:0 on using chickpeas and purslane seed oil samples. Peanut seed oil has highest long chain SFAs content [109], comprising 7.2% of C20:0 and C22:0. C22:0 percentages were found to be similar (0.60%) in both PSO and SSO seed oils (Figure 1). Our results are consistant with those obtained by other investigators [100,109], found the C20:0 content was 0.80% in pursalin seed oil using cold-pressed extract method.

**3.3. Cancer induction**

The present study was done to investigate the antioxidant and anticancer effects of PSO and SSO seed oils against DENA-induced liver carcinogénesis using male albino rats. Subcutaneous administration of this carcinogén undergoes metabolic activation in the liver to form different metabolic intermediates and these carcinogens were conversion into DENA reactive metabolites involves the activation and detoxification [62,114, 115]. Oxidative stress was involved in the process of tumour development of DENA carcinogénesis [40,63,116]. Oxidation of DNA, proteins and lipids plays an important role in a wide range of common diseases, including cardiovascular, inflammatory and cancer [19,51,66]. Many investigators [56,63,117], reported fatty acids of cell membrane is oxidized by reactive oxygen species initiates lipid peroxidation that produces free radicals, toxic substances and lipoperoxides which induces cell proliferation and contributes to cáncer (62,118].The present study is focus on more detailed on finding of new antioxidant and anticancer materials with their potential roles in cancer prevention and treatments.

Plant seeds were found to be associated with people from ancient time in food and generally consumed for its nutritive values and medicinal therapeutic properties [47,48,114], they reported these seed exhibited higher anticancer activity due to their antioxidants and polyunsaturated fatty acids contents (39,46,52]. Parsely (PSO) and [spinach](https://en.wikipedia.org/wiki/Pumpkin_seed_oil) (SSO) seed oils are mainly composed of polyunsaturated fatty acids, phenolic and flavonoids [24,36].The health benefits of these oils have been reported in the last decade and their prebiotic effects demonstrate the content of these oils depends on their constituents of polyunsaturated fatty acid and other phytochemicals (21,32,35]. Previous studies in vitro cytotoxicity test revealed that seed oils exhibited anticancer activity against different cancer cell lines due to different percentages of phytochemical and polyunsaturated fatty acid contents [4,36] they found the phytochemical, phenolic and flavonoid containing oils reduce the risk and inhibit or retard the development of carcinogenic activities. However, seed oils inhibits cell proliferation of human cancer cell lines in vitro that could arrest the cell cycle and generate apoptosis [35,118]. Moreover, anti-proliferative effects of differemt seed oils against different human cáncer cells were reported by several investigators (25,31,37]. Administration of parsley and spinach products to human is simple, since they are used as common dietary constituents in many regions of the world. Parsley seed oil (PSO) was isolated from Parsley seeds and spinach seed oil (SSO) was isolated from spinach seeds contains different percentages of phenolic, flavonoids and polyunsaturated fatty acids (Table 1). Therefore, investigation of antioxidant and anticancer activities of PSO and SSO seed oils in vivo using male albino rats were carried out at doses of 200mg/kg body weight. Diethylnitrosamine (DENA) was used as a potent and complete carcinogen for the liver, since it has been reliably used to induce liver carcinogenesis in rats after six doses over 6 successive weeks (five/week). Intraperitoneal injection of DENA (20mg/kg) five times weekly for 6 consecutive weeks induces liver cancer. Oral administration of PSO and SSO seed oils at doses of 200 mg/kg did not produce any signs of toxicity to rats and no animals were ill or died, indicate the PSO and SSO seed oils were safe and nontoxic to rats. Chemotherapy and raditherapy have poor diagnosis with some side effects [10,13] and the developing more effective and less toxic anti-cancer agents, including natural products, is necessary to prevent or retard the process of hepatocarcinogenesis [10,55]. Diethylnitrosamine (DENA) is a well establish hepatocarcinogenic agent [116,117] and the rat is the most experimental models widely used for DENA hepatocarcinogenesis study [55,56]. Rat liver is similar to that of human livers in DENA metabolize [114], generates reactive oxygen species causing oxidative stress and exhibited different changes in rat liver that are responsible for the development of hepatocarcinogenesis [4,10,118,]. Administration of the antcancer and antioxidant seed oils, have been shown to be treatment and preventive agents against DENA induced hepatocarcinoma. Antioxidant and anticancer activities of PSO and SSO seed oils used in the present study were done on chemically induced liver carcinogenesis in vivo using male albino rats. The chemical carcinogenic compound commonly used is diethylnitrosamine (DENA) as carcinogenicsubstance for inducing hepatocarcinogenic in vivo using rats [56,63,102]. Rats are widely used as experimental models to study DENA-induced hepatocarcinogenesis [55,63].The liver cáncer (hepatocarcinogenic cáncer) in the present study was induced by intraperitoneally injection of DENA at a dose of 20 mg/kg body weight five a week for 6 weeks [4]. DENA was used as carcinogen for the hepatocarcinogenesis, since it has been reliably used to induce hepatocarcinogenic after 6 doses (five/week) over 6 successive weeks [10,55]. DENA administered intraperitoneally injection in rats will metabolized by liver to generate reactive oxygen species causing oxidative stress and liver injury [7,116]. Other studies [55,118[, reported the dose of DENA (20 ml/kg body weight) five a week for 6 weeks administered to rats is found optimal for inducing toxicity,free radicals and hepatocellular carcinoma.[117,118]where it induces DNA damage, preneoplastic lesions and tumours.

**3.4. Biochemical parameters**

Data in Table (2) represented the potential effect of PSO and SSO seed oils (20mg/kg b.w.) on the levels of total protein, albumin, globulin and liver marker enzymes (ALP, ALT, AST and γ-GT) in sera of hepatocellular carcinogenic (DENA) and treated rat groups (C/PSO, C/P=SSO, PSO/C and SSO/C). Serum transaminases are considered to be sensitive indicators of liver injury in DENA-induced cancer rats where the liver was necrotized [55,62]. Liver damage induced by chronic treatment leads to liver cell necrosis and consequently elevated levels of serum transaminases [4,23,63]. The hepatic damage was indicated by marked elevation in ALP, ALT and AST levels. The degree of protection was evaluated by determining the marker enzymes (ALP, ALT and AST) and total proteins. ALP, ALT, AST are reliable markers of liver function [23,44]. Many investigators [4,23,52] studied the hepatoprotective effects induced liver damage in rats. Moreover, the increases in ALP, ALT, AST and γGT levels in rat sera were reported in cancer due to liver dysfunction [40,66]. An increase in the ALT and AST levels in plasma might be mainly due to the leakage of these enzymes from the liver into the blood stream which gives an indication of the hepatotoxic effects [44,63]. DENA exhibited various changes of biochemical parameters in liver of cárcinogenic rats group (C). Administration of PSO and SSO seed oils (200 mg/kg b.w.) showed different efects on biochemical parameters of rats group received DENA (C). Significant reductions in serum total protein, albumin and globulin levels were observed in DENA-induced liver cancer rats group (C) as shown in Table (2). Significant increases were observed in the levels of total protein (44% and49%), albumin (45% and 50%) and globulin (41% and 47%) in sera of all treated rat groups administered PSO and SSO seed oils respectively (PSO/C and SSO/C) as compared to control group C (Table 1). Significant reductions in the levels of serum protein, albumin and globulin due to inhibition of protein degradation [21] and the reduction in albumin level resulting from liver disorders [49,55,118] and decrease in albumin synthesis due to the highly toxic effect of carcinogens leads to formation of free radicals damaging proteins [21,66].

Table (2). Biochemical parameters in sera of experimental rat groups.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameters | C | C/PSO | C/SSO | PSO/C | SSO/C |
| Total protein (g/dl) | 3.90±0.10 | 5.60±0.10 | 5.80±0.10 | 5.86 ±0.10 | 5.90±0.2 |
| Albumin (g/dl) | 2.40±0.06 | 3.48±0.08 | 3.60±0.02 | 3.70±0.10 | 3.80±0.10 |
| Globulin g/dl | 1.50±0.10 | 2.12±0.10 | 2.20±0.04 | 2.16±0.04 | 2.10±0.06 |
| Alkaline phosphatase (IU/L) | 280.20±360 | 160.80±3.04 | 156.20±1.60 | 144.2.±1.90 | 128.40±1.60 |
| ALT(U/ml) | 54.80±1.20 | 34.80±1.20 | 28.60±1.20 | 26.80±1.02 | 22.98±0.80 |
| AST(U/ml) | 58.40±1.80 | 30.80±1.24 | 36.04±1.10 | 24.90±1.02 | 38.42±1.20 |
| γ-GT (U/L) | 194.42±3.16 | 116.60±2.20 | 94.20±1.98 | 84.90±1.08 | 76.90±1.04 |

Data was presented as mean value ± SE of 7 rats / group.

DENA administration to rats increased the levels of sera liver function enzymes that considered most sensitive markers in diagnosis of toxicity and hepatocellular damage [4,96,116,]. Results showed the ALP, ALT and AST levels were elevated significantly accompanied with significant decrease in protein, albumin and globulin concentrationsin DENA carcinogenic rats (C). These findings attributed to DENA that leading to malfunction of the liver [1020,,45,55,114].These results are in agreement with those reported by many investigators [40,44,59], reported significant elevations in the levels of sera ALP, ALT and AST in rat liver diseases. Similar results obtained by other investigators [23,63,66] found significant elevations in the levels of serum ALP, ALT, AST and γGT in liver diseases and disorders in hepatocellular damage caused by a number of agents including cáncer [116,118]. The present results showed significant increases in the level of ALP, ALT, AST and γ-GT in sera of rats group administered DENA (C). Higher significant decreases were observed in the levels of ALP, ALT, AST and γ-GT (Table 2) in sera of rats administered PSO and SSO seed oils as compared to those administered DENA (C). Results showed significant decreases in the levels of ALP (43 % and 44%), ALT (37% and 48%) and AST (40% and 57%) in sera of treated rat groups (C/PSO and C/SSO) with PSO and SSO seed oils respectively as compared to those of C rat group (Table 2). The present results showed higher reduction in the levels of γ-GT (40% and 52%) in sera of rat groups (C/PSO and C/SSO respectively) compared to C rat group. The value of ALT and AST activities in sera of rats received PSO and SSO seed oils reflected their improvement of liver function enzymes. A significant decrease in the levels of serum ALP by action of PSO and SSO seed oils compared to hepatotoxic rats DENA (group C) revealed the improving and protective effects of PSO and SSO seed oils on rat liver.These results are in agreement with those repoted by other investigators used different seed oils [4,23,104]. The reduction in the levels of these parameters were observed in rat groups received PSO and SSO seed oils indication of the stabilities of plasma membranes and repair of hepatic tissue damage caused by DENA. The administration of PSO and SSO seed oils showed significant decreases in serum AST and ALT activities as compared to the DENA hepatotoxic rats (C). These findings are closely related to the previous evidence [20,55,118]. Hepatic marker enzyme γGT was significant elevated in será of rats group adminstered DENA (C), indicating damage of the liver cell membrane and other changes as a result of DENA carcinogenesis [,423,63], reported the elevation in the levels of γGT in sera of DENA rats (C) cause damage liver cell membrane followed liberation of γGT from plasma membrane into the circulation of the hepatic cells as a result of carcinogenesis [23,55,,60]. However, the levels of γGT and ALT in será of rats have been used in diagnosis of primary liver cancer [20,44,98]. PSO and SSo seed oils adminstrations to rat groups prevent the increase of these hepatic enzymes, especially in rat groups received PSO and SSO seed oils before DENA adminstration (PSO/C and SSO/C groups) suggesting that the PSO and SSO seed oils have potential protective effect against DENA-induced liver cancer and may be improvement the liver from DENA injury [20,60,118]. Results also showed higher significant increase in lipid peroxidase (LP) level was observed in sera of DENA-induced liver cancer rats group (C) as shown in Figure (2). Higher significant decreases were observed in the levels of LP in sera of rats treated groups (C/PSO and C/SSO) with PSO and SSO seed oils (46% and 45% respectively). Highest reductions in the levels of LP (61% and 59%) were found in sera of rat groups given PSO and SSO seed oil before induction with DENA (PSO/C and SSO/C) compared to treated rat groups (C/PSO and C/SSO) and those of DENA control rat group (C) as shown in Figure (2).

**3.6. Anticancer and antioxidant activities of PSO and SSO**

The present data showed higher significant increases in the levels of CEA, CA15-3, CA 19-9 and CA 125 in sera of DENA-induced liver cancer rats group (C) as shown in Figure (3). CEA is used as a tumor marker for the clinical management of cancer [4,7,10,23,60], indicated that the CEA and CA19-9 tumor markers are signaling in the promotion, progression and development of cancer. CEA and CA-19.9 showed marked decrease in the rat groups treated with PSO and SSO seed oils before induction of liver DENA (PSO/C and SSO/C) more than that decrease was shown in the rat groups that treated with PSO and SSO seed oils after induction of tumors substances, DENA (C/PSO and C/SSO). Significant decreases were observed in the level of CEA, CA15-3 CA 19-9 and CA125 in sera of rat groups treated with PSO and SSO seed oil as compared to those of DENA control group C (Figure 3). A marked reduction in the level of CEA was observed in sera of rat groups received PSO and SSO seed oil (PSO/C and SSO/C) as compared to those of DENA control group C (Figure 3). These results are indicating the protective and treatment role of PSO and SSO seed oils against DENA as chemically induced liver cáncer [60,62,66]. Significant decreases were observed in the levels of CA15-3 and CA125 in sera of rat groups treated with PSO and SSO seed oils. Moreover, CEA and CA19-9 showed marked decreases in sera of rat groups received PSO and SSO seed oils before induction with DENA (PSO/C and SSO/C) more than that decreases were shown in rat groups treated with PSO and SSO seed oils after induction with DENA (C/PSO and C/SSO) as shown in Figure (3). Similar results were reported by many investigators [7, 45,55,56,66], reported protective effects of seed oils against various chemically induced liver cancer using different carcinogenic or toxic materials as carbon tetrachloride, rifampicin, and cadmium.

Antioxidant enzymes (GSH-T, GSH-P, GSH-R and SOD) consider natural defenses antioxidant for scavenger free radicals and protect cells against oxidative stress. DENA-induced liver cancer of rats showed significant decreases in the activities of GSH-T, GSH-P, GSH-R and SOD in plasma and tissue homogenates of liver, kidney and heart of DENA rats (C) as shown in Figures 4 to 7. Similar results were reported in the enhancement effect of DENA by other investigators [7,10,60,63], reported the administration of DENA to rats exhibited decreases in the levels of antioxidant enzymes. The decreased activities of GSH-T, GSH-P, GSH-R and SOD could be due to the dangerous increases in the level of free radical enhanced lipid peroxidation, inactivation of the antioxidant enzymes and detoxification of toxic DENA metabolites by tumor cells [4,19,24,62] indicated the induced chemical oxidative stress by cellular accumulation of lipid peroxides leading to decline in GSH-P, GSH-R and SOD levels. DENA cancer rats (C group) showed different percentages of decreases in the activities of GSH-T, GSH-P, GSH-R and SOD levels in liver, kidney and heart tissues of the experimental rat groups(Figures 4-5). These results are consistent with previous findings by other investigators [24,60,62], reported that such subsequent decreases in the antioxidant defense is due to the decreased expression of these antioxidants during hepatocellular damage [44,60,62]. GSH-T, GSH-P, GSH-R and SOD are defense line against reactive oxgen species due to low activity of antioxidant enzymes in some organs and oxidative stress of DENA-induction. High levels of antioxidants increase the plasma antioxidant capacity, decreasing tumor growth and inhibiting malignant cells prolifraton [4,30,39,63]. GSH-T is important antioxidant involved of cellular detoxification of endogenous and exogenous compounds and protects cells against effect of oxidative stress by scavenging free radicals and suppressing lipid peroxidation. Results showed the GSH-T activity was significant increases in plasma, liver, kidney and heart of rats treated with PSO and SSO seed oils (C/PSO and C/SSO) as shown in Figure (4). These results are similar to those obtained by other investigators [56,62,66,114].

Results showed GSH-P activity was significant increases in plasma, liver, kidney and heart of rat groups treated with PSO and SSO seed oil (C/PSO and C/SSO). These results are in accordance with tose reported by other investigators [22,24,112] stated the GSH-P reduced hydrogen peroxides and protect cell from peroxidative damage from free radical. The PSO and SSO seed oils will improve the levels of antioxidant to exert their scavenging mechanisms and exhibiting their inhibitory effects against liver carcinogenesis [18,19,25], Other studies [4,55,62,118], reported some seed oils are provide protection against earlier stages of carcinogenesis in rats. PSO and SSO seed oils played an important role as a protective factor for DENA-induced toxicity free radicals [23,24,49,114], reported the GSH-P is responsible for most of the decomposition of lipid peroxidation in cells and protect the cell from the deleterious effects of peroxidation.

GSH-P and GSH-R were significant increases in liver and kidney of rat groups (C/PSO and C/SSO) treated with PSO and SSO seed oils respectively as compared to DENA-induced liver cancer rat (C). PSO and SSO seed oils were contains antioxidant compounds, makes an effective antioxidant against DENA induced free radical generation [7,44,62]. Polyunsaturated fatty acid (PUSFAs) and phytochemical constituents of seed oils inhibits the process of carcinogenesis effectively and prevent the development of cancer in vitro and in vivo [30,36,60]. Decrease in the levels of GSH-P and GSH-R activities (Figures 5,6) during DENA toxicity might be due to antioxidant enzymes resulted during the enhanced oxidative stress and lipid peroxidation [11,25,66]. This oxidative stress is reduced by action of PSO and SSO seed oils leading to a marked increase in the activity of GSH-P (Figure 5) compared to rats administered DENA (C group) and helping to maintain liver cell integrity and control the level of liver enzymes [10,55,118]. These results are in agreement with other investigators [44,60,114], studied the effects of DMH, DENA and CCl4 on lipid peroxidation and antioxidant enzyme activities of GSH-P, GSH-R and SOD. GSH-R was significant increases in plasma, liver, kidney and heart of rat groups treated with PSO amd SSO seed oils (C/PSO and C/SSO) as shown in Figure (6). Results showed GSH-R concentration in the liver tissue was significant increase in rats treated with the PSO and SSO seed oils compared to rats group received DENA (C) as shown in Figure 6. These antioxidant activities were increased on adminstrations of PSO and SSO seed oils, which may be due to the free radical scavenging property of seed oils and consequently decreased utilization of the antioxidant enzymes [36,49,62,98]. The finding of the present results is the PSO and SSO seed oils have antioxidant and anticancer activity against DENA induced liver cancer due to higher polyunsaturated fatty acid contents that protect liver from cancer [46, 49,107],

Results of the present study indicated that PSO and SSO seed oils tend to improve the GSH-R concentrations in the rat tissues [10,24,56]. The present results showed the activities of GSH-P and GSH-R were significant increases in liver and kidney of rat treated with PSO and SSO seed oils compared to DENA- induced liver cancer rat groups (C). These results are in the same line with earlier investigation [10,23,44,118], reported the primary radicals, by donating hydrogen radicals, are reduced to non-radical chemical compounds and this action helps in protecting the body from degenerative diseases. Recent studies on the antioxidant properties of some plant materials revealed their stimulatory action on antioxidative enzymes [38,60,110], reported that the natural products induced significant increases in GSH-P and GSH-R activities and exerted a protective and antioxidant effects. Rat groups received PSO and SSO seed oils (PSO/C and SSO/C) showed increases in the activities of GSH-P and GSH-R in plasma, heart and kidney (Figures 4,5,6). Results of the present investigation showed higher activities of GSH-P and GSH-R in liver and kidney in rat groups given both seed oils (C/PSOand C/SSO) as compared to those of DENA-induced control rat group C (Figures 5, 6). SOD consider the first line of defense against free radicals derived from oxygen and lipid peroxidation [30,44,114] shows that the antioxidant substances which scavenge free radicals play an important role in the prevention of free radical-induced diseases [7,30,60]. SOD, one of the major antioxidant enzymes, decomposes superoxide peroxide, blocks lipid peroxidation and protects the tissue against oxidative damage [4,30,60,114]. In the present study the activity of SOD in liver, kidney and heart was also investigated. The present results show that the rat groups received PSO and SSO seed oils (C/PSO and C/SSO) exhibited higher SOD activity in liver and kidney as compared to those of carcinogenic control group C (Figure 7). These results are similar to those reported by other studied [44,63,66], stated imbalance between radical-generating and radicalscavenging systems produce oxidative stress. PSO and SSO seed oils showed higher increases in the activities of SOD and it scavenges superoxide radicals and reduces myocardial damage caused by free radicals [23,49,59] found similar increased in SOD activity in liver and kidney of rat groups treated with rapeseed and radish seed extracts leads to the absence of accumulation of superoxide anion radical might be responsible for decreased lipid peroxidation in these tissues [44,60,118].

This is evident from the fact that relatively higher decrease in lipid peroxidation in liver and kidney of rats given both seed oils being accompanied by the relatively higher increase in SOD activity in these tissues [25,66,112]. The findings of the PSO and SSO seed oils had antioxidant activity and protect the organs from free radicals and might be retard the progress of the diseases [4,49,98]. The present results are consistent with other investigators demonstrate alterations in the liver antioxidants in rats [25,38,44,55,118] stated a positive correlation with PSO and SSO seed oils contents and SOD scavenging activity. PSO or SSO seed oils consider natural products were found to be rich in poluynsaturated fatty acids, phenolic and flavonoid copmounds that exhibits relatively high antioxidant activity against DENA carcinogenesis. The principal agents responsible for the protective effects could be the presence of antioxidant substances that exhibit their effects as free radical scavengers [38.63].The most significant findings of the present study is that the PSO or SSO seed oils at the dose of 200 mg/kg body weight for 20 weeks have shown beneficial effect not only on liver cancer but also on antioxidant activity in DENA- induced [liver carcinogenesis in the rats](http://www.scialert.net/asci/result.php?searchin=Keywords&cat=&ascicat=ALL&Submit=Search&keyword=diabetic+rats). Moreover, PSO and SSO seed oils were ameliorated DENA-induced decrease in the activities of antioxidant enzymes. Therefore, the present results revealed the protective properties effect of PSO or SO seed oil by antagonizing DENA toxicity. The present study examined possible usefulness of PSO or SSO seed oils, as natural source of antioxidant to treat and protect the rat against oxidative stress and carcinogenic effects of DENA and improve antioxidant enzymes which can protect cell against oxidative stress of DENA. PSO and SSO seed oils are rich in polyphenols, phytochemicals and these substances are likely significant factors in the antioxidant status of health. Thus, intake of PSO and SSO seed oils in rats modified enzymatic activities and enhanced antioxidant free radical scavenging contribute to proection against cancer and other diseases.

## Inhibitory effect of PSO and SSO seed oils on hepatic enzymatic activities may be due to its acting as a hepatoprotective and ant-ilipid peroxidation agents against the permanent damage caused by DENA depending on its fatty acids and phytochemical constituents [19,36,55], including antioxidants, free radical scavenging and antiinflammatory properties preventing autoxidation and deleterious destruction of hepatic tissue [10,55]. Our findings came in harmony with other findings [59,60,63], reported the seed oils have the ability to prevent chronic diseases related to oxidative stress (cancer) and in preventing its progression due to the their higher contents of polyphenols [4,7,23,49], indicated the anti-lipid peroxidation of seed oils acted against the damaging effects of free radicals produced by DENA. SOD plays an important role in decreasing the free radicals in chemically induced liver cancer. The superoxide scavenging ability of the PSO and SSO seed oils may be due to the presence of fatty acids, phenolic and flavonoid compounds reported by other investigators [38,52,53], reported the antioxidant seed oils can be defined as a oil containing significant amounts of natural antioxidants associated to the oil [36,38,61,118] reported the fatty acids, phenolic and flavonoid compounds are important seed oils constituents that posses [antioxidant](http://www.scialert.net/asci/result.php?searchin=Keywords&cat=&ascicat=ALL&Submit=Search&keyword=antioxidant+activity) properties and play an important role as [free radical](http://www.scialert.net/asci/result.php?searchin=Keywords&cat=&ascicat=ALL&Submit=Search&keyword=free+radical) scavengers. Moreover, the increases of the antioxidant in rat groups given PSO and SSO seed oils, indicate the ability of both seed oils to prevent the formation of free radicals, enhance the endogenous antioxidant activity beyond its free radical scavenging property and the reduction of hepatic lipoperoxide formation. Thus, the present study is of preclinical trials may be helpful to develop functional foods, novel antioxidant and anticancer containing drugs used for cancer treatments and protection against chemically-induced hepatocellular carcinoma (DENA) and other various diseases.

**CONCLUSION**

The present study was done to evaluate the effects of parsely (PSO) and spinach (SSO) seed oils against DENA induced liver carcinogénesis. PSO and SSO seed oils appeared to be an effective free radical scavenging with antioxidant activities and inhibiting oxidative stress. Results suggest that the ability of PSO and SSO seed oils to ameliorate DENA-induced cancer is associated with its antioxidant and free radical scavenging properties owing their antioxidant and anticancer activities. PSO and SSO seed oils could protect rat liver from altered hepatic functioning, and improvements liver tissues. The experimental findings of this study indicate that administration of PSO and SSO seed oils effectively regulates the antioxidant defenses, inhibition the biotransformation enzymes and cause elevation in some enzymes which augments the detoxification. PSO and SSO seed oils not only natural product but also ingredients for pharmaceutical products inexpensive for clinical use, may be considered as protective agents against many cáncer types and other diseases.

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