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Research Article

Anticancer and Antioxidant Effects of *Brassica napus* and *Raphanus sativus* Seed Oils against Chemically Induced Liver Cancer in Rats

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Received: January 19, 2022; **Published:** February 22, 2022

Abstract

Rapeseed (*Brassica napus*) and radish (*Raphanus sativus*) seed oils prepared previously were used in the present study. The present study aims to investigate the antioxidant and anticancer activities of rapeseed and radish seed oils against diethylnitrosamine (DENA)-induced liver carcinogenesis in male albino rats. 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 rapeseed and radish 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 rapeseed and radish seed oils as compared to DENA control rats groups, indicating protective effects of both seed oils against harmful and toxicity of DENA. Significant decreases in the levels of lipid peroxidase (LP) in sera of rats administered rapeseed and radish seed oils compared to those of DENA control rats. Higher significant decrease in the level of LP was observed in sera of rats administered rapeseed seed oil more than those given radish seed oil. Rapeseed and radish seed oils showed more effective for inhibiting DENA-induced liver cancer through evaluation and determination of tumor markers (CEA, CA19-9, CA15-3 and 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 rapeseed and radish seed oils as compared to those of DENA control rats. The most significant findings of the present study are the rapeseed and radish 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 as well as protect cell against DENA oxidative stress by antagonizing DENA toxicity's. These finding suggested that the seed oils (200 mg/kg b.w.) could be a potential compounds used in protective and treatments of liver tumors. According to these observations, the use of R1 and R2 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 cancer in rats indicates these seeds could be used as food, food purposes, pharmaceutical and drugs for treatment of different diseases. Supplemented diets with rapeseed and radish seeds containing antioxidant and treatment agents effectively used in the chemotherapeutic treatment of different cancer types particularly liver cancer. Thus, oils can be used as strong sources of novel anticancer and antioxidant drugs for cancer treatments. Oil seed crops are use edible oil products increases with population increases and consider natural sustainability indicators due to its high efficient and productivity. In addition, this manuscript provide many ways to sustainable developments as seed oil production consider a role for sustainable production of different types of oils from different crops resulting sustainable manner for higher gain of economic from the plant seed oil crops. Seed oils contain different phytochemicals and fatty acids have antioxidant properties resulting sustainable manner for nutrition and pharmaceutical industries and in medicine for treatment of cancer and other diseases.

Keywords: *Rapeseed; Radish; Seed Oils; Anticancer; Antioxidant; Rat*

Introduction

Cancer is a global epidemic disease causing abnormal growth of the body cells that invade and destroy the normal cells causing death all over the world. Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries due to the lack of diagnostic techniques, standard methods of treatment, and higher cost of the treatments [1,2] they demonstrated the developing countries have more number of cancer incidence cases compared to the developed countries. Liver diseases have become a global concern worldwide [3]. Liver is an abdominal largest sensitive organ play effective roles in metabolism, detoxification and excretion of toxins or harmful molecules [4] reported the most of drugs ingested orally pass through the liver and metabolized into intermediates toxic with adverse side effects represented the liver injuries. Liver cancer represents a great public health problem as the 6th most common cancer in developed countries [5,6] and is the third leading cause of cancer mortality worldwide due to arising the cases rate in the last decades [2]. Liver cancer is a malignant neoplasm of hepatocytes considered the cancer of liver caused by oxidative stress and inflammation cause cancer-related mortalities [3,7,8] reported the liver cancer was arises in the setting of chronic liver disease [9,10]. Chemotherapy and radiotherapy used in cancer treatment were more expensive and have induced drug resistance that cause toxic not only to tumor cells but also to healthy cells leading to certain side effects [10,11]. The high cost increasing drug resistance and side effects of current therapeutic approaches are forcing the scientists to find and produce new compounds or drugs from plant sources for cancer treatment [12,13]. Considering the continuing need for effective inexpensive anticancer agents, several researchs concentrates for production of bioactive compounds [11,12] and novel anticancer drugs development from natural products with potential antitumour and chemopreventive activities [8].Various parts of several plants have been common among people and pharmaceutical industry were found extensively utilized in food and medical industries to synthesize and produce drugs of biological activity in the treatment of most diseases [13]. Plant-derived extracts were used as a potential treatment for cancers and induce apoptotic cell death of human colon and hepatoma cells *in vitro* and *in vivo* [4,14,15]. Plant-derived compounds as polysaccharides, alkaloids, saponins, triterpenes, polyphenols and flavonoids have shown antioxidant and anticancer properties *in vitro* and *in vivo* [14,16,17] and have wide applications in cancer therapeutics due to lower cost and induce lesser side effects compared to synthetic drugs [10,18,19]. Plant derived anticancer drugs such as paclitaxel, vinblastine, vincristine and colchicine have been approved effective anticancer drugs and are widely used in clinical practice against the most cancer types [10,20] reported different derived compounds are used in structure of new anticancer drugs development. Natural compounds, extracted from plants were used in developing novel chemopreventive compounds for cancer therapeutic strategies that could overcome limitations of conventional therapies [18] reported the anticancer drugs from natural products have low cost and exhibited several effective actions of chemotherapy against resistant cancer cells. Moreover, many studies suggested certain plant materials as natural product might be useful as anticancer and chemopreventive agents in a variety of bioassay systems and animal models due to phytochemical constituents [14,18,20]. Human carcinogenesis and chronic diseases were resulting effects of diets, nutrition and oxidative stress exhibited reduction of antioxidant defenses against cancer cells that consider the main factor in development of most cancer types [8,21]. Other investigators [16,22] indicated the diets included high fruits and vegetables containing some phytochemicals provide cancer chemoprevention and reduce the risk in developing of chronic diseases including many types of cancer by interfering with cell cycle and inducing apoptosis [13,23,24]. Phytochemicals exhibit antitumor activities through improvement the defences of antioxidant enzymes, remove oxidative stress, followed inhibition of carcinogenesis and direct absorb the reactive oxygen species [16,24,25]. Several studies indicated that the plant-derived materials containing phytochemicals could be used in preventive strategies to reduce the risk and inhibit or retard the development of cancers [11,20,22]. Dietary phytochemicals include phenolic compounds and polyunsaturated fatty acids are widely distributed in fruits and vegetables [14,25,26], may contribute to health-promoting effects through powerful antioxidant properties, decrease metastasis, induce apoptosis, and inhibit cell proliferation [27,28]. Many anticancer drugs used in medicine are derived from natural sources of fruits and vegetables involving phenolic, flavonoids and polyphenols as different kinds of antioxidants are scavengers of free radicals and are modulated during carcinogenesis or after tumor formation [17,21]. Many natural compounds such as terpenoids, phenolic, flavonoids and lignans were discovered from plant sources as antioxidant substances capable of scavenging free

superoxide radicals, protecting biological system against harmful effects of oxidative processes and play an important role in cancer treatment [14,17,28,29]. Other workers [14,16,17] reported the natural compounds with antioxidant activity can target tumor cells after disease occurrence, directly inhibit cell proliferation and prevent tumor recurrence or metastasis. Other investigators [12,27,29] stated the antioxidant compounds have anti-inflammatory, antitumor and anticarcinogenic activities. Anticancer activities of antioxidants as inhibit cancer cell proliferation, differentiation, induce apoptosis, metastasis and interfere in angiogenesis were also established [27,29]. Reactive oxygen species and or free radical consider the main factor in the formation of lipid peroxidation that consequently damage the cell membrane resulting from toxicity leads to hepatic dysfunction and reduced the glutathione responsible for removing free radicals [8,30]. Plant seeds as natural source, considered as a part of human culture used by ancient peoples were showed increases during the last decade due to its contents of many chemical ingredients used as food, feed or in medicine [14,22,29]. Plant seeds have rich nutritional and nutraceutical components used for protection against some diseases [13,31,32] reported the consumption of pumpkin and flax seed was associated with potential health benefits such as reduction of cancer risk and atherosclerosis. Recent researches [7,14,15,27] provided evidence that the consumption of some constituents of plant seed results in treated and protection against chemically induced colon and hepatocellular carcinoma. Moreover, seed extracts showed anticancer and pharmacological effects *in vitro, in vivo* and in medical trials [14,20,26]. Among natural seed extracts, oils extracted from seeds of different plant family's were found to have nutritional quality used as edible oil food ingredients in various food items and consumed in appreciable amounts in most diets [22,32,33]. Oils are biological mixtures of plant origin consisting of mixtures from glycerol and chain of fatty acids [14,26,32] 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 [34], cardiovascular and other various diseases [15,35]. Oils extracted from plant seeds have antimicrobial, antifungal, antitumor and cytotoxic activities [14,26,29]. Plant 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 [13,22]. Some seed oils have anticancer properties, nutritional quality and health benefits were evidence by other investigators [12,22,26]. Seed oils can be considered as bioactive molecules in medicine have been demonstrated to have antitumor [14] and chemopreventive effects [17,18]. Seed oils were found to be used as antiviral, antibacterial, anticancer and antioxidant agents [17,29,35,36]. Oils of different plant seeds have been shown the potential health impacts in preventing some diseases including cancer and have anti-inflammatory [36], antiproliferative [14,17], antigenotoxic [27] and anticancer activities [11,17,14] when they were used certain seed oils in cancer therapy against tumors development. Seed oils have higher anticancer ingredients, including fatty acids, phenolic and flavonoid as antioxidant compounds being associated with improved human health [5,14,17]. Seed oils with their constituents of fatty acids and phytochemicals possess various bioactivities including cytotoxicity [14,24-26], anticancer [7,25] and antidiabetic [34]. Seed oils were found to be used in treated and protection against chemically induced colon and hepatocellular carcinoma using rats [7,20,25,37] reported some plant seed oils containing phytochemicals and antioxidant compounds were beneficial to protect the mucosa against chemical carcinogenesis and protect the liver against lipid peroxidation impairment in antioxidant status induced by CCl_4 [5,37,38]. Synthetic antioxidants addition to the oils and foods were considered one of the most efficient ways of lipid peroxidation inhibition have some undesirable side effects [16,18]. Oils extracts, have become interesting in recent years and represent an alternative to synthetic antioxidant agents in food and pharmaceutical industries, alternative medicine and natural therapy [38,39]. Recently, there is growing interest in studies of natural healthy antioxidants to finding natural antioxidants replace synthetic compounds in applications [14,39,40]. Natural compounds with antioxidant activity can directly inhibit cell proliferation and stimulate the immune system [17,27]. Various studies have focused on natural antioxidant compounds that are able to delay or inhibit the oxidation of lipids or other biomolecules, and thus prevent the damage of cells caused by reactive oxygen [41-43]. Other researchers [14,21,30] reported the primary role of antioxidants is to prevent or delay oxidative lipid damage produced in proteins and nucleic acids by reactive oxygen species, including reactive free radicals. Moreover, antioxidant properties are involved in protection of the liver tissue against hepatotoxin-induced toxicity [5,28,30] they reported the antioxidants compounds inhibit the activity of the free radicals in cells and tissue, especially on the liver giving a hepatoprotective effect on the liver cells [4,17,21,37]. Rapeseed (*Brassica napus*) and radish (*Raphanus sativus*) are commonly used as

food or in medicine and consider a good sources of diet health-promoting ingredients contains fatty acids, phenolic and flavonoid compounds have biological properties, including anticancer activities [14,15,33,39]. In previous studies, rapeseed and radish seed oils were extracted using cold pressed extraction method [44], yielded, 20.8% and 14.2% respectively [26]. Fatty acid contents of rapeseed and radish seed oils were estimated and identified [26,45]. Fatty acid analyses of the obtained rapeseed and radish seed oils revealed the presence of highest levels of unsaturated fatty acids (94.4% and 91% respectively) but the saturated fatty acids represent 5.6% and 9.0% of total rapeseed and radish seed oils respectively [26]. Higher levels of linolenic acid (ω-3), linoleic acid (ω-6) and oleic acid (ω-9), consider the main constituents of unsaturated fatty acids were found in rapeseed and radish seed oils than that of vegetable seed oils [35,40] reported these fatty acids play important roles in human health and diseases prevention. Phenolic and flavonoids contents of rapeseed and radish seed oils were also estimated [26]. Interestingly, rapeseed and radish seed oils were showed anticancer and cytotoxic effects on various cancer cell lines including liver carcinoma cell lines (HEPG2) *in vitro* [26]. However, the properties of many plants and plant derived compounds particularly it's cytotoxic, anticancer and antioxidant activities have not yet been fully investigated. Moreover, to the best of our knowledge, there is a little research on rapeseed and radish seed oils antioxidant activity or anticancer effect on chemically induced hepatocellular carcinogenesis *in vivo*. Therefore, in this study, we tested rapeseed and radish seed oils antioxidant and anticancer activities against DENA-induced liver cancer *in vivo* using rats. The present research produce oils from rapeseed and radish seeds using cold pressing easily method done on crops encourages a sustainable for industries to produce natural oils and a sustainable effective way to raise economic of seed oil crops.

Materials and Methods

Materials

Diethylnitrosamine (DENA) was purchased from Sigma-Aldrich® chemie, Gmbh, Riedstr, 2, D-89555 Steinheim, Germany. All other chemicals used in the present study were obtained from Sigma Chemical Company (Sigma-Aldrich), Steinheim, Germany.

Rapeseed (*Brassica napus*) and radish (*Raphanus sativus*) seed oils were prepared previously and their chemical analyses revealed the presence of different percentages of fatty acids, phenols and flavonoids compounds [26].

In vivo studies

Cancer induction

Induction of liver cancer experimentally in rats was done [7,37] using diethylnitrosamine (DENA).

Animals and experimental design

Thirty five male albino rats, 8 weeks of age, weighing about 140 ± 1.6g were purchased from the National Research Center for biological products. The rats were randomly divided into five groups (7 rats/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 ± 5.0%. 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 [7,26,37]. One rat group administrated DENA was maintained without any treatment over experimental period (20 weeks) and used as liver carcinogenic control rat group (C). Other two rat groups from 3 groups of rats administrated DENA for 6 weeks (five/week) were then treated with daily oral doses (200 mg/kg body weight) of rapeseed (R1) and radish (R2) seed oils (C/R1 group and C/R2 group respectively) from week 7 till the end of experimental period (20 weeks). Remaining two groups of rat were administrated

daily with oral doses (200 mg/kg body wt) of R1 and R2 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 (200 mg/kg body weight) of R1 and R2 seed oils (R1/C group and R2/C group respectively) from week 7 till the end of experimental period (20 weeks).The experimental protocol was done according to the method described previously [46].

Samples preparation

At the end of experimental period (20 weeks), blood samples were drawn from 7 rats per each group separately using capillary tubes, centrifuged at 4000xg for 10 minutes. 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 minutes at 4ºC and the resultant supernatants were stored at - 70ºC till used. Stored sera or plasma and tissues homogenates were 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. Livers of rats were removed and used for pathological examinations.

Biochemical parameters

Alkaline phosphatase (ALP) level was carried out referring the Germany DGKC indications [47]. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured using kits of QCA, Spain [48]. Gamma glutamyl transferase (γ-GT) was carried out according to the kinetic colorimetric method using Biodignostic kits [49], Egypt. Total protein was estimated using Biodignostic kits, Egypt [50]. Serum albumin level was measured according to the method of Doumas., *et al* [51]. Globulin was calculated by subtracting albumin form the total protein [52]. 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 [53,54]. GSH-R (EC1.6.4.2) activity was also assayed [55]. SOD (EC 1.15.1.1) activity was measured as described by Elstner., *et al* [56]. Lipid peroxidase (LP) was also estimated [57]. 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* [58]. Carbohydrate antigens (CA 19-9 and CA 15-3) and cancer antigen 125 (CA 125) were performed with commercially available Enzyme Immunoassay Kit [59].

Histopathology

Histopathological assessments of liver tissues were carried out using Hematoxylin and Eosin (H&E) staining technique [60].

Statistical analysis

Data from the biochemical analysis was statistically analyzed using student T-test [61].

Results and Discussion

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 [31,32], they reported these seed exhibited higher anticancer activity due to their antioxidants and polyunsaturated fatty acids contents [26,29,30,35]. Rapeseed (R1) and radish (R2) seed oils are mainly composed of polyunsaturated fatty acids, phenolic and flavonoids [26]. 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 phytochemicals

[13,22,25]. *In vitro* cytotoxicity test revealed that the R1 and R2 seed oils exhibited anticancer activity against different cancer cell lines due to different percentages of phytochemical and polyunsaturated fatty acid contents [14,32], they found the phytochemical, phenolic and flavonoid containing oils reduce the risk and inhibit or retard the development of carcinogenic activities. R1 and R2 seed oils inhibits cell proliferation of liver (HEPG2) human cancer cell lines [26] that could arrest the cell cycle and generate apoptosis, which explains the *in vitro* anti-proliferative effects [17,25]. Moreover, anti-proliferative effects of different seed oils against different human cancer cells were reported by other investigators [17,27]. Chemotherapy and radiotherapy have poor diagnosis with some side effects [9] and the developing more effective and less toxic anti-cancer agents, including natural products, is necessary to prevent or retard the process of hepatocarcinogenesis [5,7,8,37].

Cancer induction

Chemical carcinogenic compound commonly used is diethylnitrosamine (DENA) as carcinogenic substance for inducing hepatocarcinogenic *in vivo* using rats [37,43]. 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 (20 mg/kg) five times weekly for 6 consecutive weeks induces liver cancer. Rats are widely used as experimental models to study DENA-induced hepatocarcinogenesis [42,43]. Rat liver is similar to that of human livers in DENA metabolize [62], generates reactive oxygen species causing oxidative stress and exhibited different changes in rat liver that are responsible for the development of hepatocarcinogenesis [7,8,63]. However, DENA is a well establish hepatocarcinogenic agent [37,64] and the rat is the most experimental models widely used for DENA hepatocarcinogenesis study [37,65]. The liver cancer (hepatocarcinogenic cancer) 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. 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 [7,16,37]. DENA administered intraperitoneally injection in rats will metabolized by liver to generate reactive oxygen species causing oxidative stress and liver injury [5,8,42]. Other investigators [37,65,66] 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 [64,66] where it induces DNA damage, preneoplastic lesions and tumours. Administration of rapeseed and radish products to human is simple, since they are used as common dietary constituents in many regions of the world. Rapeseed oil (R1) isolated from rapeseed seed and radish oil (R2) isolated from radish seed contains different percentages of phenolic, flavonoids and polyunsaturated fatty acids and have appreciable cytotoxic and anticancer activity against liver carcinoma cell line (HEPG2) *in vitro*. Therefore, investigation of antioxidant and anticancer activities of R1 and R2 seed oils *in vivo* using male albino rats were carried out at doses of 200 mg/kg body weight. Oral administration of R1 and R2 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 R1 and R2 seed oils were safe and nontoxic to rats.

Biochemical parameters

Antioxidant and anticancer activities of R1 and R2 seed oils used in the present study were done on chemically induced liver carcinogenesis *in vivo* using male albino rats. Biochemical parameters particularly serum transaminases are considered to be sensitive indicators of liver injury in DENA-induced cancer rats where the liver was necrotized [15,41,43,67]. Liver damage induced by chronic treatment leads to liver cell necrosis and consequently elevated levels of serum transaminases [15,67,68]. The hepatic damage was indicated by marked elevation in ALP, ALT and AST levels [26,69,70]. 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 [14,20,68,69]. Many investigators [35,69] studied the hepatoprotective effects induced liver damage in rats. Moreover, the increases in ALP, ALT, AST and γGT levels in sera of rats were reported in cancer due to liver dysfunction [28,46,70]. 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 [16,43,68].

Parameters	C	C/R1	C/R2	R1/C	R2/C
Total protein (g/dl)	3.40 ± 0.10	5.06 ± 0.16	5.30 ± 0.10	5.26 ± 0.14	5.44 ± 0.10
Albumin (g/dl)	1.90 ± 0.08	2.78 ± 0.08	2.98 ± 0.04	2.90 ± 0.10	3.10 ± 0.08
Globulin g/dl	1.50 ± 0.10	2.28 ± 0.08	2.32 ± 0.06	2.36 ± 0.06	2.34 ± 0.04
ALP (IU/L)	254.80 ± 5.10	140.44 ± 3.04	126.04 ± 2.60	122.10 ± 2.86	108.2 ± 2.60
ALT(U/ml)	56.90 ± 2.40	32.40 ± 1.40	30.52 ± 1.40	30.40 ± 0.90	24.40 ± 0.60
AST(U/ml)	62.80 ± 2.60	34.60 ± 2.04	32.64 ± 1.14	28.94 ± 0.94	22.04 ± 1.10
γ-GT (U/L)	178.20 ± 4.06	104.22 ± 2.04	92.14 ± 2.14	86.80 ± 1.02	74.40 ± 0.90

Table 1: *Biochemical parameters in sera of experimental rats.*

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

Data in the present study (Table 1) showed the DENA administration to rats increased the levels of sera liver function enzymes that considered most sensitive markers in diagnosis of toxicity and hepatocellular damage [5,37,69]. Results showed the ALP, ALT and AST levels were elevated significantly accompanied with significant decrease in protein, albumin and globulin concentrations in DENA carcinogenic rats (C). These findings attributed to DENA that leading to malfunction of the liver [37,41,43] obtained significant elevations in the levels of ALP, ALT and AST in sera of DENA-induced rats. These results are in agreement with those reported by many investigators [28,71,72], reported significant elevations in the levels of sera ALP, ALT and AST in rat liver diseases.

Similar results obtained by other investigators [60] 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 cancer [70]. ALP has been markedly increased by action of DENA administration causing hepatic dysfunction and reflects hepatocellular injury [42,43,63,66]. ALP levels were elevated due to defective hepatic excretion or increased production of ALP by hepatic parenchymal cells [69,70]. Administration of the anticancer and antioxidant seed oils have been shown to be treatment and preventive agents against DENA induced hepatocarcinoma. Data in table 1 represented the potential effect of R1 and R2 seed oils (20 mg/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. DENA exhibited changes of biochemical parameters in the liver of cancer rats group (C). Administration of R1 and R2 seed oils (200 mg/kg) showed differences in effects on biochemical parameters of DENA-induced hepatic cancer rats group (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 1. These results are in agreement to those reported by other studies [43,46] reported reduction in albumin level resulting from liver disorders and decrease in albumin synthesis due to the higher toxic effect of DENA carcinogens leads to formation of free radicals damaging proteins [63,64,71]. Significant increases were observed in the levels of total protein (49% and 56%), albumin (52% and 57%) and globulin (52% and 56%) in sera of treated rat groups administered R1 and R2 seed oils respectively (R1/C and R2/C) as compared to control group C (Table 1). These results are similar to those reported by other investigators [14,43]. Highest significant increases were observed in the levels of total protein (55% and 60%), albumin (53% and 63%) and globulin (57% and 55%) in sera of rat groups administered R1 and R2 seed oils respectively (C/R1and C/R2 groups) as compared to treated and carcinogenic control groups (Table 1). 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 in sera of rats administered R1 and R2 seed oils as compared to those administered DENA (C). Results showed significant

decreases in the levels of ALP (45% and 51%), ALT (43% and 46%) and AST (45% and 48%) in sera of treated rat groups (C/R1 and C/R2) with R1 and R2 seed oils respectively as compared to those of C rat group. Higher reduction were observed in the levels of ALP (52% and 58%), ALT (47% and 57%) and AST (54% and 64%), in sera of rat groups (R1/C and R2/C) given R1 and R2 seed oils respectively as compared to C rat group. Treatment of DENA carcinogenic rats with R1 and R2 seed oils reduced the activities of ALP and ALT in plasma and consequently alleviated liver damage caused by chemical-induced cancer. The value of ALT and AST activities in sera of rats received R1 and R2 seed oils reflected their improvement of liver function enzymes. A significant decrease in the levels of serum ALP by action of R1 and R2 seed oils compared to hepatotoxic rats DENA (group C) revealed the improving and protective effects of R1 and R2 seed oils on rat liver. These results are in agreement with those reported by other investigators used different seed oils [5,69,70]. In the present study, higher decrease in the levels of ALP, ALT, AST and γGT activities accompanied with significant increase in albumin concentration were observed in rat groups received R1 and R2 seed oils (R1/C and R2/C) than those of rats treated with R1 and R2 seed oils (C/R1 and C/R2) in comparing to DENA rat group (C). The reduction in the levels of these parameters were observed in rat groups received R1 and R2 seed oils indication of the stabilities of plasma membranes and repair of hepatic tissue damage caused by DENA. These results are in accordance with those reported by other investigators [41,42,43,69]. The administration of R1 and R2 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 [64.66]. Hepatic marker enzyme γGT was significant elevated in sera of rats group administered DENA (C), indicating damage of the liver cell membrane and other changes as a result of DENA carcinogenesis [43,70]. Elevation in the levels of γGT in sera of DENA rats (C) cause damage liver cell membrane [42,43,70], followed liberation of γGT from plasma membrane into the circulation of the hepatic cells as a result of carcinogenesis [14,15,26,43]. The present results showed higher reduction in the levels of γ-GT (42% and 48%) in sera of rat groups (C/R1 and C/R2 respectively) compared to C rat group. A marked reduction in the levels of γ-GT (51% and 58%) was observed in sera of rat groups given R1 and R2 seed oil (R1/C and R2/C respectively) as compared to C rat group (Table 1). R1 and R2 seed oils administrations to rat groups prevent the increase of these hepatic enzymes, especially in rat groups received R1 and R2 seed oils before DENA administration (R1/C and R2/C groups). These results suggesting that the R1 and R2 seed oils have potential protective effect against DENA-induced liver cancer and may be improvement the liver from DENA injury [91,98]. However, the levels of γGT and ALT in será of rats have been used in diagnosis of primary liver cancer [68,69]. Results also showed higher significant increases in lipid peroxidase (LP) levels were observed in sera of DENA-induced liver cancer rats group (C) as shown in figure 1. Similar results obtained by other investigators [4,37,66] they reported the DENA effects of lipid peroxidation. Significant decreases were observed in the levels of LP in sera of rats treated groups (C/R1 and C/R2) with R1 and R2 seed oils (49% and 55% respectively) as compared to those administered DENA control rat groups (C). These results are in accordance with those reported by other investigators [42,46] indicated the decreased lipid peroxides by cellular accumulation resulting from carcinogen oxidative stress. Higher reductions in the levels of LP (62% and 68%) were found in sera of rat groups given R1 and R2 seed oil before induction with DENA (R1/C and R2/C) compared to those of treated rat groups (C/R1 and C/R2) and DENA control rat group (C) as shown in figure 1. These results are in accordance with those reported by other investigators [24,26,71]. Highest significant decreases in the levels of LP (55% and 68%) were observed in sera of rats administered R2 seed oil (C/R2 and R2/C) more than those of rats groups (C/R1 and R1/C) received R1 seed oil (49% and 62%inistered (R1/C and R2/C) as compared to treated rat groups (C/R1 and C/R1) and those of DENA control rats group (C) as recorded in Table (1). Reduction in the levels of LP activity against DENA toxicity may be due to the R1 and R2 seed oils antioxidant that prevent the formation of lipid peroxidation [8,41,68]. R1 and R2 seed oils have antioxidants scavenging free radicals and suppressed lipid peroxidation that protects cells against effect of DENA oxidative stress [14,73].

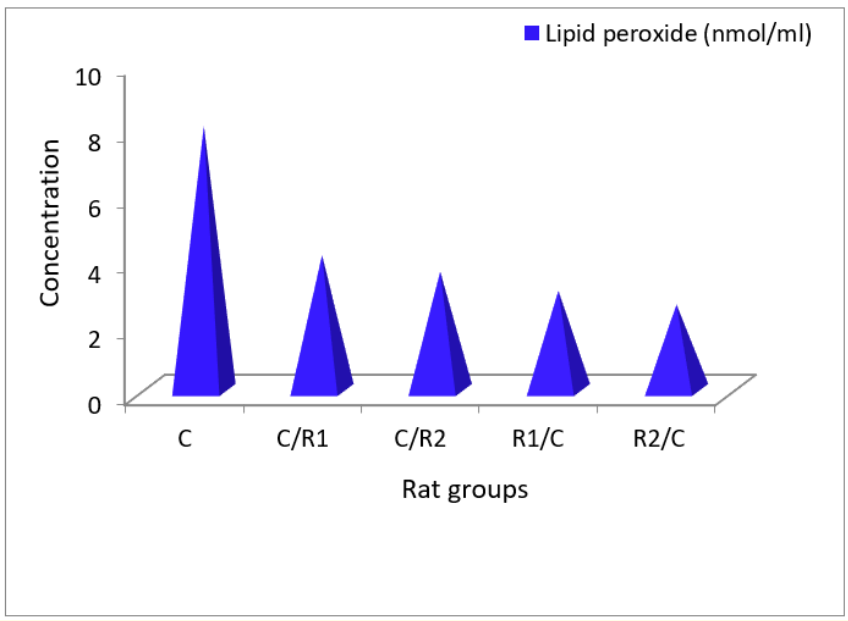

Figure 1: *Lipid peroxidase activities in sera of experimental rats.*

Tumor markers

Measurements of CEA, CA19-9, CA15-3 and CA125 tumor markers in the present study were done before treatment and after 20 weeks of R1 and R2 seed oils treatments. These results are in line with those reported by other studies [74] they were indicated that the measurements of these tumor markers, were performed before treatment and after 4, 6 and 36 months of treatment, showed statistically significant decreases during this periods. Results revealed significant increase in the levels of CEA, CA19-9, CA15-3 and CA125 tumor markers in sera of DENA rat groups (C) as shown in figure 2. The values of the tumor markers CA 19-9 and CA 125 were elevated usually detected in chronic, gallbladder and granulomatous liver diseases [74,75] while the elevated values of 15-3 were found in chronic hepatitis as nonmalignant diseases [74]. Significant decreases were observed in the level of CEA and CA 19-9 in sera of rat groups treated with R1 seed oil (C/R1) and R2 seed oil (C/R2) respectively, as compared to those of DENA control group C (Figure 2). Taheri., *et al*. [75] indicated that the CEA and CA19-9 tumor markers are signaling in the promotion, progression and development of cancer. A marked reduction in the level of CEA was observed in sera of rat groups received R1 and R2 seed oil (R1/C and R2/C) as compared to those of DENA control group C (Figure 2). Significant decreases were observed in CA19-9 in sera of rat groups treated with R1 and R2 seed oils (Figure 2). Slightly decreases were observed in the levels of CA 15-3 and CA125 in sera of rat groups given R1 and R2 seed oils. CEA and CA-19.9 showed marked decrease in the rat groups treated with R1and R2 seed oils before induction of liver DENA (R1/C and R2/C) more than that decrease was shown in the rat groups that treated with R1 and R2 seed oils after induction of tumors substances, DENA (C/R1 and C/R2). Results also showed decreases in the levels of CA 15-3 and CA 125 in sera of rat groups given R1 and R2 seed oils [74-76].

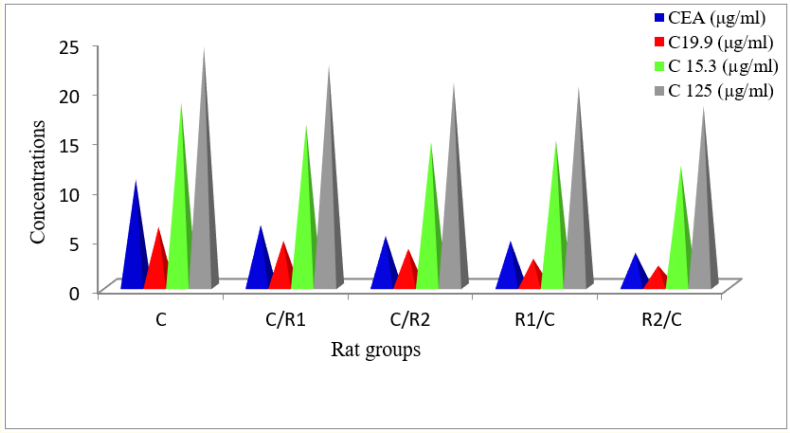

Figure 2: *CEA, CA 19.9, CA 15.3 and CA 125 levels in sera of experimental rats.*

Moreover, all rat groups received R1 and R2 seed oils before and after DENA tumors induction are markedly decreased as compared to carcinogenic (DENA) control rat groups (C). CEA, CA 125 and CA-19.9 have many biological aspects as adhesion, fibrosis, metastasis and apoptosis [26,46,75]. These results are indicating the protective and treatment role of R1 and R2 seed oils against DENA as chemically induced liver cancer [26,46]. Many investigators [5,34,35,38,53,72] 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 activities

The present study, focused on investigating the antioxidant and anticancer effects of R1 and R2 seed oils against DENA-induced liver carcinogenesis using male albino rats. Subcutaneous administration of this carcinogen 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 [42,62]. Oxidative stress was involved in the process of tumour development of DENA carcinogenesis [28,43]. Oxidation phenomena have been implicated in many illnesses, such as diabetes mellitus, arteriosclerosis and cancer. Oxidation of DNA, proteins and lipids plays an important role in a wide range of common diseases, including cardiovascular, inflammatory and cancer [12,34,46]. Other investigators [38,43,64] 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 cancer [42,66]. Antioxidant enzymes as GSH-T, GSH-P, GSH-R and SOD consider natural defenses antioxidant scavenger free radicals and protect cells against harmful oxidative stress. DENA has significantly decreased in the activities of GSH-T, GSH-P, GSH-R and SOD in plasma and tissues homogenates of liver, kidney and heart of rat group C (Figure 3-6 respectively). 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). Similar results were reported in the enhancement effect of DENA by other investigators [5,7,14,43] 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. Other studies [12,16,42] indicated the induced chemical oxidative stress by cellular accumulation of lipid peroxides leading to decline in GSH-P levels. In the present study, the DENA cancer rats (C) 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. These results are

consistent with previous findings by other investigators [16,42,73] reported that such subsequent decreases in the antioxidant defense is due to the decreased expression of these antioxidants during hepatocellular damage [30,68]. GSH-T, GSH-P, GSH-R and SOD are defense line against reactive oxygen 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 proliferation [21,24,37]. The present results (Figure 3) showed the activity of GSH-T was significant increases in plasma (37% and 46%), liver (38% and 50%), kidney (27% and 32%) and heart (45% and 50%) of rat treated with R1 and R2 seed oils (C/R1 and C/R2). 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 [38,73]. GSH-P (Figure 4) was significant increases in plasma (55% and 58%), liver (51% and 63%), kidney (48% and 59%) and heart (38% and42%) of rat treated with R1 and R2 seed oil (C/R1 and C/R2). GSH-P has a high potency in scavenging reactive free radicals in response to oxidative stress and detoxifies peroxides [8,14,37]. R1 and R2 seed oils were contains antioxidant compounds, makes an effective antioxidant against DENA induced free radical generation [5,42,68]. Polyunsaturated fatty acid and phytochemical constituent of seed oils inhibits the process of carcinogenesis effectively and prevent the development of cancer *in vitro* and *in vivo* [21,26,40]. GSH-P reduced hydrogen peroxides and protect cell from peroxidative damage from free radical [14,34,46]. R1 and R2 seed oils improve the levels of antioxidant to exert their scavenging mechanisms and exhibiting their inhibitory effects against liver carcinogenesis [12,17] they reported the seed oils are provide protection against earlier stages of carcinogenesis in rats. Several studies [8,26,33] showed R1 and R2 seed oils played an important role as a protective factor for DENA-induced toxicity free radicals. Similar results were obtained by other investigators used different seed oils [14,37,42,66]. Moreover, the inhibition of peroxidation by seed oils is mainly attributed to the scavenging of the reactive free radical involved in the peroxidation and disturbing the antioxidant leading to oxidative stress and carcinogenesis [21,30,66]. GSH-R (Figure 5) was significant increases in plasma (50% and 57%), liver (55% and 60%), kidney (40% and 45%) and heart (37% and 44%) of rat treated with R1 and R2 seed oils (C/R1 and C/R2). In the present study, the results showed that, GSH-R concentration in the liver tissue was significantly higher in rats treated with the R1 seed oil (C/R1 and R1/C) and R2 seed oil (C/R2 and R2/C) than in rats received carcinogenic materials, DENA (C2). These antioxidant activities were increased on administrations of R1 and R2 seed oils, which may be due to the free radical scavenging property of seed oils and consequently decreased utilization of the antioxidant enzymes. Other workers [26,30,33,37] stated the free radical scavenging and anticarcinogenic properties of R1 and R2 seed oil has been associated with their bioactive compound contents [42]. Similar results were obtained by other investigators [26,33,41] finding the rapeseed (R1) and radish (R2) seed oils have antioxidant and anticancer activity against DENA induced liver cancer due to higher polyunsaturated fatty acid contents that protect liver from cancer [14,26]. Results of the present study indicated that R1 and R2 seed oils tend to improve the GSH-R concentrations in the rat tissues. These results are in agreement with those reported by other investigators [7,14,16,38].

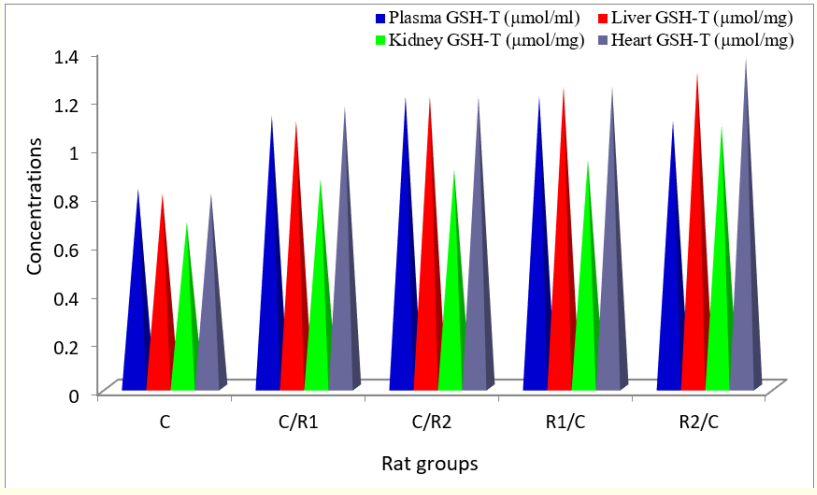

Figure 3: *GSH-T levels in plasma, liver, kidney and heart of experimental rats.*

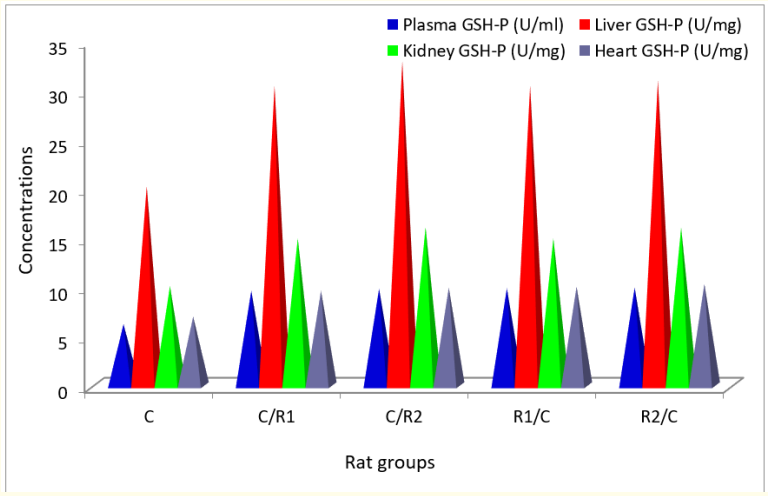

Figure 4: *GSH-P levels in plasma, liver, kidney and heart of experimental rats.*

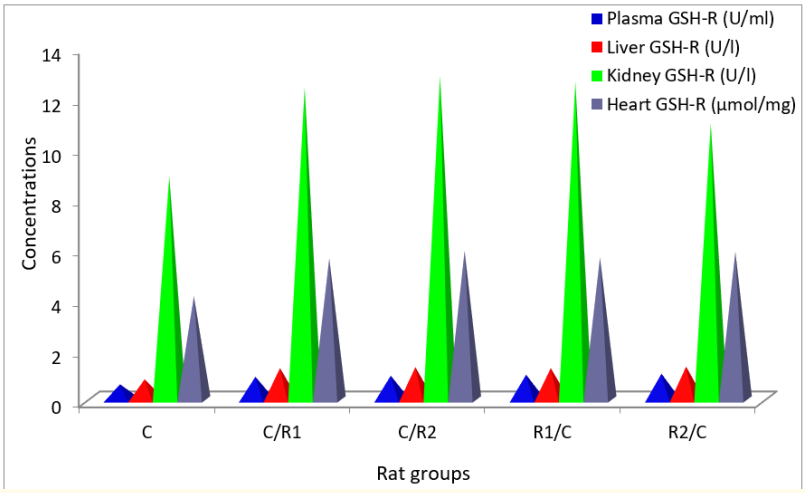

Figure 5: *GSH-R levels in plasma, liver, kidney and heart of experimental rats.*

Data in the present study showed the activities of GSH-P and GSH-R were significant increases in liver and kidney of rat groups (C/R1 and C/R2) treated with R1 and R2 seed oils respectively as compared to DENA-induced liver cancer rat (C). Rat groups received R1 and R2 seed oils (R1/C and R2/C) showed higher significant increases in the activities of GSH-P (59% and 60%) and GSH-R (63% and 70%) in plasma (Figure 4 and 5), Rats received R1 and R2 seed oils (R1/C and R2/C) showed increase in the activities of GSH-P (43% and 48%) and GSH-R (38% and 43%) in heart (Figure 4). GSH-R activity was increased in liver (55% and 63%) and kidney (43% and 66%) in rat groups (R1/C and R2/C respectively) compared to those of C rats (Figure 5). GSH-P activity was also increased (Figure 4) in liver

(51% and 53%) and kidney (49% and 59%). GSH-T activity was also increased in liver (55% and 63%) and kidney (38% and 59%) as shown in figure 3. GSH-P is responsible for most of the decomposition of lipid peroxidation in cells and may thus protect the cell from the deleterious effects of peroxidation. Results of the present investigation showed higher activities of GSH-P and GSH-R in liver and kidney in rat groups given seed oils (C/R1and C/R2) as compared to those of DENA-induced control rat group C (Figure 4 and 5). In heart tissue, however, enhanced lipid peroxidation in rats may be due to lower effect of DENA on the levels of GSH-P and GSH-R activities shown in rat group C (Figure 4 and 5).

Decrease in the levels of GSH-P and GSH-R activities during DENA toxicity might be due to antioxidant enzymes resulted during the enhanced oxidative stress and lipid peroxidation [8,17]. This oxidative stress is reduced by action of R1 and R2 seed oils leading to a marked increase in the activity of GSH-P compared to rats administered DENA (C group) and helping to maintain liver cell integrity and control the level of liver enzymes [7,37,66]. These results are in agreement with other investigators studied the effects of DMH, DENA and CCl4 on lipid peroxidation and antioxidant enzyme activities of GSH-P, GSH-R and SOD. The present results showed the activities of GSH-P and GSH-R were significant increases in liver and kidney of rat treated with R1 and R2 seed oils compared to DENA- induced liver cancer rat groups (C). These results are in the same line with earlier investigation [7,26,37]. Moreover, 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 [15,68,72]. Recent studies on the antioxidant properties of some plant materials revealed their stimulatory action on antioxidative enzymes [40,69] reported that the natural products induced significant increases in GSH-P and GSH-R activities and exerted a protective and antioxidant effects. Other studies demonstrated decreases in GSH-P activity and alterations in liver antioxidants of rats [5,16,41]. Results obtained from the present study are very much promising and similar to the observation reported in streptozotocin induced diabetic rats [34,46]. In the present study the activity of SOD in liver, kidney and heart was also investigated. SOD, one of the major antioxidant enzymes, decomposes superoxide peroxide, blocks lipid peroxidation and protects the tissue against oxidative damage [16,21,76,77]. Generally, free radicals are produced in the body as the result of metabolic processes. The imbalance between radical-generating and radical scavenging systems produce oxidative stress [43,68,71]. Free radicals are the source of lipid peroxidation derived from oxygen and SOD is the first line of defense [21,26,37]. SOD consider the first line of defense against free radicals derived from oxygen and lipid peroxidation [21,68,73] shows that the antioxidant substances which scavenge free radicals play an important role in the prevention of free radical-induced diseases [5,21,69]. The principal agents responsible for the protective effects could be the presence of antioxidant substances that exhibit their effects as free radical scavengers [41,43,71]. Results in the present study show the rat groups received R1 and R2 seed oils (C/R1and C/R2) exhibited significant increase in the activity of SOD of liver (59% and 65%) and kidney (36% and 50%) respectively as compared to those of carcinogenic control group C (Figure 6). Rats received R1 and R2 seed oils (R1/C and R2/C) showed higher significant increases in the activities of SOD in liver (61% and 66%) and kidney (50% and 61%) respectively as compared to those of control rat group C (Figure 6). R1 and R2 seed oils showed higher increases in the activities of SOD and it scavenges superoxide radicals and reduces myocardial damage caused by free radicals [14,15,33,39] 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 [68,71,76]. 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 [17,26,46]. Other investigators [21,33] findings the R1 and R2 seed oils had antioxidant activity and protect the organs from free radicals and might be retard the progress of the diseases. These results are consistent with other investigators demonstrate alterations in the liver antioxidants in rats [22]. From these results, it appeared that there was a positive correlation with R1 and R2 seed oils contents and SOD scavenging activity [37,38]. Inhibitory effect of R1 and R2 seed oils on hepatic enzymatic activities may be due to its acting as a hepatoprotective and antlipid peroxidation agents against the permanent damage caused by DENA depending on its fatty acids and phytochemical constituents [12,14,38,76] including antioxidants, free radical scavenging and anti-inflammatory properties preventing autoxidation and deleterious destruction of hepatic tissue [7,37]. Our findings came in harmony with other studies [39,40] reported the rapeseed (R1) and radish (R2)

seed oils have the ability to prevent chronic diseases related to oxidative stress, such as cancer and in preventing its progression due to the their higher contents of polyphenols. Anti-lipid peroxidation of R1 and R2 seed oils were found to be acted against the damaging effects of free radicals produced by DENA [15,33]. SOD plays an important role in decreasing the free radicals in chemically induced liver cancer.

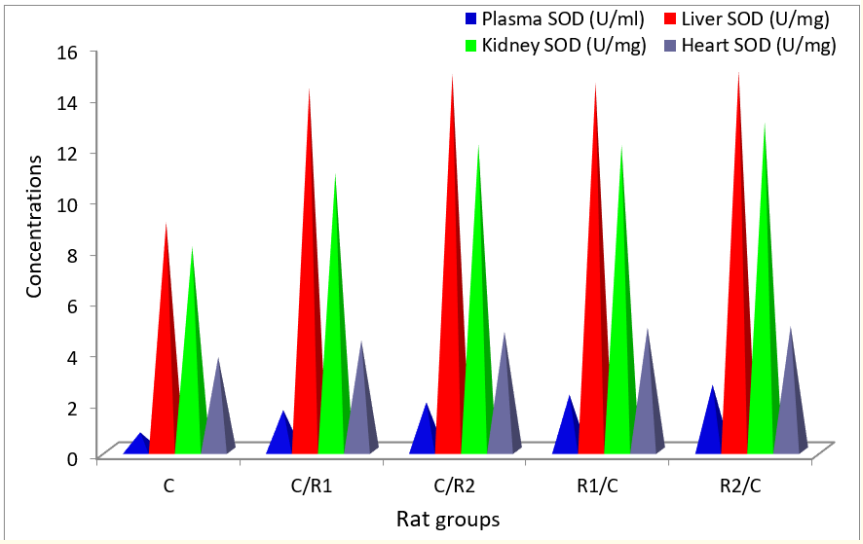

Figure 6: *SOD levels in plasma, liver, kidney and heart of experimental rats.*

In the present study, combined treatment of DENA with R1 or R2 seed oils resulted in maintained the activities of SOD and GSH-dependent antioxidant enzymes, when compared to C control group, indicating the protective role of R1 or R2 seed oils against DENA-induced oxidative stress. R1 or R2 seed oils consider natural products were found to be rich in polyunsaturated fatty acids, phenolic and flavonoid compounds that exhibits relatively high antioxidant activity against DENA carcinogenesis.

The superoxide scavenging ability of the R1 and R2 seed oils may be due to the presence of fatty acids, phenolic and flavonoid compounds reported by other investigators [26,35] reported the antioxidant seed oils can be defined as an oil containing significant amounts of natural antioxidants associated to the oil [41]. Other investigators [14,26,41] reported the fatty acids, phenolic and flavonoid compounds are important seed oils constituents that possess antioxidant properties and play an important role as free radical scavengers. However, the chemical properties of R1 and R2 seed oils in terms of their availability as radical scavengers predict their antioxidant activity [14,26]. The most significant findings of the present study is that the R1 or R2 seed oils at the dose of 200 mg/kg body weight for 20 weeks have shown beneficial effect not only on liver cancer *in vitro* and *in vivo* but also on antioxidant activity in DENA- induced liver carcinogenesis in the rats. Moreover, R1 and R2 seed oils were ameliorated DENA-induced decrease in the activities of antioxidant enzymes. Therefore, the present results revealed the protective properties effect of R1 or R2 seed oil by antagonizing DENA toxicity. The above findings suggested the R1 and R2 seed oils have potent free radical scavenging and antioxidant activities in DENA induction for hepatocellular carcinogenesis. Our data suggest that the ability of R1 and R2 seed oils to ameliorate DENA liver injury is associated with its antioxidant and reactive oxygen scavenging properties.

The present study examined possible usefulness of R1 or R2 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. Antioxidant capacity depends on redox and free radical scavenging characteristics of the medium along with the content of several antioxidant compounds. R1 and R2 seed oils are rich in polyphenols, phytochemicals, and these substances are likely significant factors in the antioxidant status of health. Thus, intake of R1 and R2 seed oils in rats modified enzymatic activities and enhanced antioxidant free radical scavenging contribute to protection against cancer and other diseases. Moreover, the increases of these antioxidant in rat groups given R1 and R2 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 and novel antioxidant and anticancer containing drugs used for cancer treatments and protection against chemically-induced hepatocellular carcinoma (DENA) and other various diseases.

Histopathology

Examined sections of liver from DENA rats group (C) revealed necrosis and the damaging effect of DENA used in the form of marked fibrosis with cellular infiltration (arrow) around the main blood vessels specially in the portal area. Dilatation with congestion of blood sinusoids and vacuolar degeneration of hepatocytes were observed in upper right corner as shown in figure 7a. These findings are in the same line with [28] recorded the histopathological examination of DENA rat liver showing large focal area of hepatocellular necrosis infiltrated with mononuclear inflammatory cells. Sections of liver tissue from rat group (C/R1) received treating R1 seed oil (Figure 7b) showing slight decrease in fibrosis, although cellular infiltration is still present (arrow). The lower right corner (Figure 7b) shows a local aggregation of cellular infiltrate at a higher magnification. Sections of liver tissue from rat group (C/R2) received treating R2 seed oil (Figure 7c) showing in the left part of the figure a remarkable reduction of fibrosis and cellular infiltration. Dilatation and congestion of blood sinusoids is still noticed (arrow). The right side of the figure 7c is a higher magnification showing mild vacuolar degeneration of hepatocytes, marked reduction of fibrosis and cellular infiltration around blood vessels and a local aggregation of cellular infiltrates less than that observed in the previous sections (Figure 7a and 7c) of DENA control group and those of treated rat group with R1 seed oil respectively. However, sections of liver tissue isolated from C/R1 and C/R2 treated rat groups showed decrease in lymphoid, normal mucosal lining and the lamina propria showed minimal inflammatory cells. Dilatation and congestion of blood sinusoids is still present (Figure 7b and 7c). Sections of liver tissue from a rat group (R1/C) received R1seed oil before induction with DENA (Figure 7d) showing blood sinusoids dilatation (arrow) especially at the periphery of lobules (near portal areas) and focal aggregations of cellular infiltration (arrowhead). The upper right part (Figure 7d) showed higher magnification of a part of the section showing a band of fibrous tissue with cellular infiltration. Sections of liver tissue from a rat group (R2/C) received R2 seed oil before induction with DENA (Figure 7e) showing great decrease of fibrosis and cellular infiltration except at portal area, which shows dilated and congested portal vein (arrow). The upper right part (Figure 7e) shows mild dilatation of blood sinusoids and more or less normal hepatocytes at a higher magnification. Photomicrograph section of liver tissue from rat used in the present study showing great decrease of fibrosis and cellular infiltration at portal area in section in liver of rat group (R2/C) received R2 seed oil than the liver of rat group (R1/C) received R1 seed oil. R2 seed oil showing great decrease of fibrosis and cellular infiltration at portal area in sections of rat liver than that of rats received R1 seed oil. Results indicated the sections of liver tissue from a rat received R1 and R2 seed oils before DENA administrations (R1/C and R2/C groups) showing disappearance of lymphoid and cellular infiltration all over the tissue although mild blood sinusoids dilatation and congestion were observed. Submucosa, mucosa and serosa are within normal with no pathological changes (Figure 7d and 7e). These results are in agreement with those findings reported by other investigators [7,15,26,37].

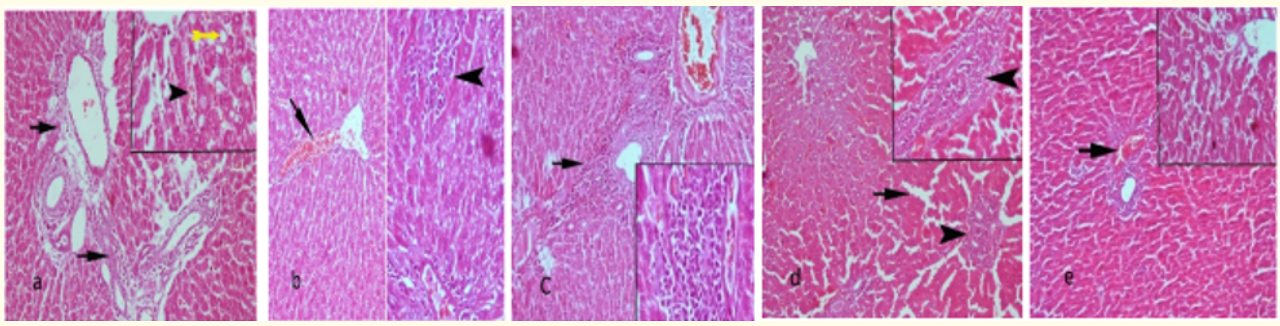

Figure 7: *Sections of DENA-induced liver cancer rats group (a) and treated rats groups (b, c, d, e).*

These results suggested the important use of R1 and R2 seed oils as protective agents against DENA carcinogenesis. Moreover, these histopathological studies indicated the R1 and R2 seed oils (200 mg/kg) showed great improves in the histology of liver tissues in rats received DENA carcinogens. The present study examined possible usefulness of R1 or R2 seed oil separately or in mixing together, as natural antioxidants treated and protected rats from carcinogenic effects of DENA carcinogens and improve hepatic and antioxidant defense enzymes that protect the cells from harmful oxidative stresses and lipid peroxidation. Histopathological studies indicated that the use of R1 and R2 seed oils (200 mg/kg) showed improved in the histology of rats received DENA carcinogens. Moreover, results suggested the important roles of R1 and R2 seed oils as protective agents against harmful tissues of carcinogenesis. However, based on the published studies, administration of R1 and R2 seed oils to man is simple, since, they are used as common dietary constituents in many parts of the world. Rapeseed (R1) and radish (R2) seed oils rich in fatty acids and phytochemicals have been used in treated tumours in medicine indicated to production of suitable new pharmaceutical therapeutic drugs used in low doses for protective and treatment of different human cancers and encourage as nontoxic natural products. According to these observations, the present study establishes that the R1 and R2 seed oils have appreciable anticancer and antioxidant activities. Further studies including clinical trials with advanced techniques are required for oils or their constituents to be more effective, targeted and specific treatments and inhibition of carcinogenesis. The results of the present study provides oil seed crops cultivation, production and role for sustainable production of different types of natural oils from different crops resulting sustainable manner for higher income through production of biofuel used in various applications and more sustainable source of fuel than petroleum. Glycerol as a by-product used extensively in food and cosmetics industries in different areas of the world. Moreover, the presence of phytochemical and polyunsaturated fatty acids in seed oils makes important oil seed crop due to potential health effects.

Conclusion

The present study was done to evaluate the effects of Rapeseed (R1) and radish (R2) seed oils against DENA induced liver carcinogenesis. R1 and R2 seed oils appeared to be an effective free radical scavenging with antioxidant activities and inhibiting oxidative stress. Results suggest that the ability of R1 and R2 seed oils to ameliorate DENA-induced cancer is associated with its antioxidant and free radical scavenging properties owing their antioxidant and anticancer activities. Results revealed the potent anticancer efficacy of R1 and R2 seed oils as natural products by attenuating the metabolic and hepatic enzymes disorders. R1 and R2 seed oils could protect rat liver from altered hepatic functioning, and improvements liver tissues. The experimental findings of this study indicate that administration of R1 and R2 seed oils effectively regulates the antioxidant defenses, inhibition the biotransformation enzymes and cause elevation in some enzymes which augments the detoxification. These activities were assessed based on biochemical parameters, antioxidant enzymes level

in liver, kidney and heart homogenates and histopathological observations. R1 and R2 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. Moreover, the present study establishes that R1 and R seed oils as natural products containing fatty acids and phytochemicals have appreciable anticancer and antioxidant activities. Seed oils consider major sources of sustainable and raw materials that increasing income all over the world. Therefore, increasing oil production of seed oil crops is thus essential for more sustainable development in the future.

Acknowledgment

The authors acknowledge the National Research Center, Dokki, Cairo, Egypt, for providing research necessary facilities.

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