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Antioxidant and Anticancer Activities of *Carthamu stinctorius* and *Portulaca oleracea* Seed Oils *In Vitro* and *In Vivo* Using DMH-Induced Colon Cancer Rats

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Abstract

Safflower (*Carthamus tinctorius*) and purslane (*Portulaca oleracea*) seed oils prepared previously were used in the present study. The present study was done to investigate the antioxidant and anticancer activities of Safflower (SSO) and purslane (PSO) seed oils *in vitro* and *in vivo* using 1,2 Dimethylhydrazine (DMH)-induced rats. DMH induced rats showed significant changes in biochemical parameters and increases the levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in sera of rats. Significant decreases were also found in the activities of antioxidant enzymes defense in plasma and tissues of rats. Glutathione transferase (GSH-T), glutathione peroxidase (GSH-P), glutathione reductase (GSH-R) and superoxide dismutase (SOD) activities were reduced in plasma and tissues of DMHinduced rat group. A marked reduction were observed in the levels of ALP, ALT and γ-GT in sera of rat groups given SSO and PSO exhibited improving in biochemical changes of these liver function enzymes, indicating the role effects of both seed oils against harmful and toxicity of DMH. Significant decreases were observed in the levels of lipid peroxidase (LP) in sera of rats administered SSO and PSO. Higher significant decrease in the level of LP was observed in sera of rats administered SSO more than those given PSO. Safflower (SSO) and purslane (PSO) seed oils showed more effective for inhibiting DMH-induced colon cancer through evaluation and determination of tumor markers (CEA, CA19-9, CA15-3 and CA125) in sera of DMH-induced colon carcinogenic rats group 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 SSO and PSO. The most significant findings of the present study are the Safflower (SSO) and purslane (PSO) seed oils have shown beneficial effect not only on colon cancer but also on antioxidant defense enzyme activities in DMH- induced colon carcinogenesis in rats as well as protect cell against DMH oxidative stress by antagonizing DMH toxicity. According to these observations, the use of SSO and PSO 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 colon carcinogenesis. Thus the present study suggest the possibility of produced many types of inexpensive seed oils, have shown beneficial effects on chemically induced colon cancer in rats, indicates these seeds could be used as food, food purposes, pharmaceutical and drugs for treatment of different diseases.

***Keywords**: Seed Oils; Anticancer; Antioxidant; In Vitro; In Vivo; Rats*

Introduction

Plant and some various parts of several plants have been common among people and pharmaceutical industry used for production of drugs used for treatment of several diseases [1-3]. Safflower (*Carthamus tinctorius*) and Purslane (*Portulaca oleracea*) were wildly distributed in different areas of the world used as industrial ingredients for production of functional food and therapeutic agents. Several investigators evidence the pharmacological and medicinal effects of edible safflower and purslane plants such as antibiotic [4], antioxi-

dant [5], hypoglycemic [6], antibacterial [7] and antitumor activities [8]. 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 [9-11] reported plant seeds have rich nutritional and nutraceutical components used for protection against some diseases [12]. In last decades cancer cases were raised in different areas all over the world specially in developing countries where higher cost of treatment drugs, side effects of current therapeutic, conventional diagnostic techniques, behavior life of peoples and environmental conditions [13,14] reported cancer is a global epidemic disease causing abnormal growth of the body cells that invade and destroy the normal cells causing death in both developed and developing countries. Different types of cancer diseases infect and inherent human but the colorectal cancer are being the most common forms [15]. Colon cancer represents a great public health problem that consider the third most common cause of death in different parts of the world with higher rate of morbidity and mortality due to unsuccessful treatments using traditional therapy [16]. Most of the modern chemotherapeutic and radiotherapeutic agents have been reported to exhibit severe toxicity to normal tissues and resistance of cancer cell, accompanied by undesirable side effect [17]. Increasing concern drug resistance, undesirable side effect and the expensive of current drug used in cancer treatments leads to researcher of therapeutic field are focusing to find other sources for production of novel natural therapeutic agents used in treatment of cancer [1,18]. Some studies found anticancer properties of many compounds isolated from different plants used in cancer therapeutics and more effective than synthetic drugs [1,3,8,15,18]. Bioactive compounds and novel anticancer drugs development were heavily produced from natural products to meet the continuing need for these anticancer drugs against cancer cases increases [19-21]. Anticancer drugs were development from natural products with potential anti-tumour and chemopreventive activities [1,18,20]. Moreover, different plant-derived anticancer drugs such as vinblastine, vincristine, colchicine have been approved effective anticancer drugs and are widely used in clinical practice against the most cancer types [1,18,22] reported different derived compounds are used in structure of new anticancer drugs development. Different studies exhibit the various effects of natural products [1,10,19,21] evidence the biological activities of natural products such as antioxidant, antimicrobial and anti-inflammatory. Other investigators studies the anticancer and antioxidant properties of natural products as polysaccharides, alkaloids, saponins, triterpenes, glycosides, polyphenols and flavonoids *in vitro* and *in vivo* [7,20,22] reported these compounds have anti-inflammatory and anticarcinogenic effects. Some natural products were found to be used in structures and development of cancer chemotherapy drugs [1,20,23]. Natural products have been regarded as important sources of potential chemotherapeutic for developing novel chemopreventive compounds for cancer therapeutic strategies that could overcome limitations of conventional therapies [1,24] reported the anticancer drugs from natural products have low cost and exhibited several effective actions of chemotherapy against resistant cancer cells. Numerous studies reported some compound, containing phytochemical constituents extracted from plants, showed cancer prevention and chemoprevention against tumor development [20,24]. Other studies indicated the phytochemicals compound containing products used for prevent and delay cancer development [7,12,25]. Consumption of pumpkin and flax seed was associated with potential health benefits such as reduction of cancer risk and atherosclerosis [3,6,7]. Moreover, seed extracts showed anticancer and pharmacological effects *in vitro*, *in vivo* and in medical trials [1,18,19]. Among natural seed extracts, oils extracted from seeds of different plant families were found to have nutritional quality used as edible oil food ingredients in various food items and consumed in appreciable amounts in most diets [2,3,11]. Oils extracted from plant source contains glycerol and fatty acids chains [15,20] reported these fatty acids are important for growth, biological, biochemical and physiological changes responsible for human health. Antioxidant properties of natural compound extracted from plant seeds were studied and showed cytotoxic and cell proliferation inhibition, resulting stimulate the immune system [10,20,26] they stated the antimicrobial, antifungal and antitumor effects of oil extracts as natural compounds. Oil consumption in certain amounts could be improved the biochemical changes resulting oxidative stress and decreases risk of most diseases [27]. Oils contains some bioactive molecules showed cytotoxicity [19], anticancer [15], antimicrobial, antiviral and antioxidant [7,10] and chemopreventive in cancer therapy [1,20,25] they were investigated mustard oil effect against tumor development [26]. Other studies were stated the oils have effect on diabetes [6], cardiovascular and some chronic diseases [12,13,21]. Experimental studies were confirmed the non-toxic effect of seed oils when used as ingredients for functional food and pharmaceutical productions [3,11,21,26].

Other investigators reported the anticancer properties, nutritional quality and health benefits of certain seed oils as chickpeas and safflower supplemented diets [3,11,15,28]. Seed oils of different plants have been shown the potential health impacts in preventing some diseases and have anti-inflammatory activities [21] and anticancer activities [20]. Antiproliferative activities of different seed oils against different diseases were reported by other investigators [7,9,19] when they were used certain seed oils in cancer therapy against tumors development. Seed oils with their constituents of fatty acids and phytochemicals possess various bioactivities including cytotoxicity [5,25,26]. 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 [3,27,29]. Many studies [9,11], 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 and inducing apoptosis [3,9,12]. Free radicals produced in the body resulting of the metabolic processes are responsible for various human diseases and 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 [29,30]. Different compounds were used as natural antioxidants were obtained from plant sources such as terpenoids, phenolic, flavonoids and lignans have wide application in drugs used for treatment of cancer types [5,19,25,29]. Natural compounds with antioxidant activity can target tumor cells after disease occurrence, scavenging free superoxide radicals directly inhibit cell proliferation and prevent tumor recurrence or metastasis [3,9,19]. Other investigators found anti-inflammatory, antitumor and anticarcinogenic activities of antioxidant compounds resulting improving the health state of humans [10,20,21]. Phytochemicals and fatty acids, are the major constituents of seed oils, exhibits antitumor activities through improvement the defenses of antioxidant enzymes, remove oxidative stress, followed inhibition of carcinogenesis and direct absorb the reactive oxygen species [30-32] reported these seed oils were used for inhibit or retard cancer development [11,20,21]. Dietary phytochemicals include phenolic compounds and polyunsaturated fatty acids are widely distributed in fruits and vegetables [11,26,33], may contribute to health-promoting effects through powerful antioxidant properties, decrease metastasis, induce apoptosis, inhibit cell proliferation and stimulate the immune system [9,13,21]. Oil seed crops are use edible oil products increases as the population increases in recent years and consider natural sustainability indicators due to its high efficient and productivity used for replace synthetic antioxidant used in food and pharmaceutical industries [1,3]. Natural antioxidants are the most important discovery and are increasingly used for replacing synthetic antioxidants and lipid peroxidation inhibition of food industry [31-33] fond some natural products exhibit biological, antioxidant and anti-inflammatory activities [10,15,21]. Seed oils have higher anticancer ingredients, including fatty acids, phenolic and flavonoid as antioxidant compounds being associated with improved human health [3,7,33] 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 carcinogen. In previous studies, we were used the cold press extraction method [33,34] and have extracted safflower (*Carthamus tinctorius*) and purslane (*Portulaca oleracea*) seed oil yields (32.8% and 18.2 respectively). Safflower and purslane seed oils were analyzed, identified and found these seed oils contains saturated and unsaturated fatty acids [26,33,34]. Safflower and purslane seed oils contain different percentages of linolenic, linoleic and oleic acids [3,7,26,33,34]. Linolenic, linoleic and oleic acids in different seed oils were obtained by other investigators [15,33,34]. Palmitic, stearic and arachidonic acids were found in safflower and purslane seed oils with different variations [28,30]. Phenolic and flavonoids contents were detected in safflower and purslane seed oils [26,29]. However, the quantity and chemical composition of seed oil varies depending on different conditions. Hence the present study has been made to investigate the antioxidant and anticancer activities of safflower (*Carthamus tinctorius*) and purslane (*Portulaca oleracea*) seed oils *in vitro* and *in vivo* using DMH- induced colon cancer rats.

Aim of the Study

The aim of the study was to identify the antioxidant and anticancer properties of the safflower and purslane seed oils. Cytotoxic effect and anticancer activities of both seed oils against HCT116 carcinoma cell line *in vitro* were done. In addition, seed oils contain different phytochemicals and fatty acids have antioxidant properties resulting sustainable manner for food, pharmaceutical and medical industries.

Materials and Methods

Seed oils

Safflower (*Carthamus tinctorius*) and purslane (*Portulaca oleracea*) seed oils were prepared previously using cold-pressed extraction process at low temperature [9,33], Safflower (SSO) and purslane (PSO) seed oils were kept in dark bottles and stored at -18°C till used.

Colon cancer cell line

Colon cancer cell line (HCT-116) was obtained from the National Cancer Institute, Cairo University, Egypt.

Carcinogenic material

1, 2 dimethylhydrazine dihydrochloride 99+% (DMH) was obtained 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.

In vitro studies

Antioxidant activity

DPPH (2,2-diphenyl-1-picryhydrazyl) radical scavenging activity assay the scavenging activity of DPPH free radicals was measured [35]. Results are expressed as the amount of sample necessary to scavenge 50% of DPPH radicals (IC_{50}).

Anticancer activity

Safflower (SSO) and purslane (PSO) seed oils were investigated *in vitro* against HCT-116 colon carcinoma cell line [36] with different concentrations (0, 12.5, 25, 50 and 100 mg/ml). The experiment was repeated three times for each oil sample separately.

Cytotoxicity

Cytotoxicity tests of both oil samples obtained were assessed against the HCT-116 as the human tumor cell lines [36]. IC50 was calculated by analysis between surviving fraction and oil samples concentration [35,36].

In vivo studies

Cancer induction

Induction of colon cancer experimentally in rats was done using 1, 2 dimethyl hydrazine (DMH) according to the previous method described [37].

Animals and experimental design

Twenty eight male albino rats, 8 weeks of age, weighing about 160 ± 1.1g were purchased from the National Research Center for biological products. Rats had free access to fed commercial diet and tap water. 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. The rats were randomly divided into four groups (7 rats/group) were housed in a wire screen cage. Three groups of rats were administrated for 5 weeks (twice/week) subcutaneous injections of 1,2-dimethyl-hydrazine (DMH) at a dose of 40 mg/kg body weight [37]. One group of the three groups was maintained without any treatment over experimental period (16 weeks) and used as colon carcinogenic control group (C). Two groups of rats administrated DMH for 5 weeks (twice/week) were then treated with oral dose (200 mg/kg body weight) of SSO

and CSO (C/SSO group and C/PSO group respectively) from week 6 till the end of experimental period (16weeks). The experimental protocol was done according to the method previous described [37]. All animals' procedures were performed in accordance with the ethical guidelines and policies approved by the Ethics Committee of NRC (2013).

Samples preparation

At the end of experimental period (16 weeks), blood samples were drawn from 7 rats per each group separately using capillary tubes, centrifuged at 4000xg for 10 minutes and 20 minutes using cooling centrifuge (Sigma 2K15). 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 precyzyjnawarszawa model MPW-309, Poland). The homogenates were centrifuged at 15,000xg for 20 minutes at 4ºC using cooling centrifuge (Sigma 2K15) 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 transferase (GSH-T), glutathione peroxidase (GSH-P), glutathione reductase (GSH-R), superoxide dismutase (SOD) and other biochemical parameters.

Biochemical parameters

Alkaline phosphatase (ALP) was carried out referring the indications, Germany [38]. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured using kits of QCA, Spain [39]. Gamma glutamyl transferase (γ-GT) was estimated according to the kinetic colorimetric method [40] using Biodignostic kits, Egypt. Total protein was estimated using Biodignostic kits, Egypt [41]. Serum albumin level was also measured [42]. Globulin was calculated by subtracting albumin form the total protein [43]. Glutathione transferase (EC 2.5.1.18), GSH-T Glutathione peroxidase (EC1.11.1.9) activities (GSH-P) in plasma and homogenates of liver, kidney and heart tissues were assessed [44,45]. Glutathione reductase (EC1.6.4.2) activity (GSH-R) was assayed [46]. Superoxide dismutase (EC 1.15.1.1) activity (SOD) was measured by the NADH oxidation procedure [47]. Lipid peroxidase (LP) was estimated [48]. Determination of carcinoembryonic antigen (CEA) was performed with commercially available Enzyme Immunoassay Kit [49]. Carbohydrate antigens (CA 19-9 and CA 15-3) and cancer antigen 125 (CA 125) were performed with commercially available Enzyme Immunoassay Kit [50].

Statistical analysis

Data from the present study were statistically analyzed using student T-test [51].

Results and Discussion

In vitro studies

Antioxidant of SSO and PSO

Antioxidant activity is determined *in vitro* assays using DPPH radical scavenging activity [35]. Results showed the DPPH scavenging activities of SSO and PSO were increased with the increased oil concentrations (Figure 1). Results showed the antioxidant activities of SSO and PSO were detected at concentrations of 4 mg/mL and 6 mg/ml respectively. IC_{50} values of DPPH radicals scavenging of SSO and PSO were 10.4 mg/ml and 14.2 mg/ml respectively. DPPH free radical scavenging of SSO was higher than that of PSO [29]. These effect may be attributed the different contents of fatty acids between the two seed oils particularly linolenic and palmitic acids in SSO. These results are higher than those reported by other investigators [3,26,32]. The variations in these results may be due to higher percentage of linolenic acid content in SSO than PSO. Results of the present study are in agreement with those reported by other studies [5,7,28]. The higher DPPH radical scavenging activity of SSO and PSO maybe due to its higher content of linolenic acid [3,5,29,34]. Phenolic and flavonoid compounds represents strong antioxidants were found in SSO and PSO, have scavenger the free radicals [5,12,24,25].

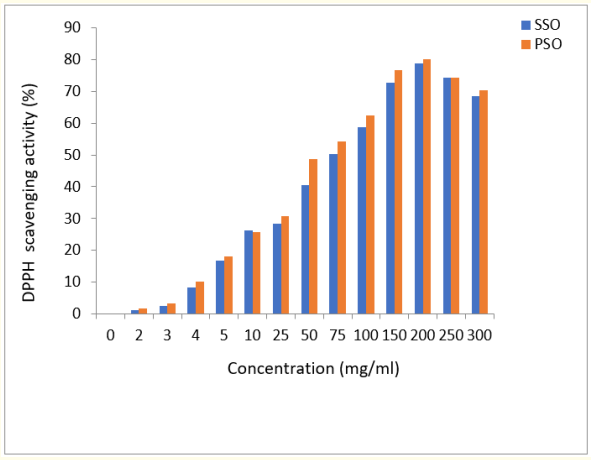

Figure 1: *Antioxidant activities of SSO and PSO in vitro.*

Cytotoxicity test of SSO and PSO

The potential effects of SSO and PSO against colon carcinoma cell lines (HCT-116) *in vitro* (Figure 2) using *in vitro* cytotoxicity test. Results showed that SSO was more effective on growth inhibition of HCT-116 carcinoma cell line than that of PSO. The effects of both seed oils are inhibit the cell proliferation of human colon cancer cell line (HCT-116) *in vitro*. Similar results were obtained [21,26]. SSO and PSO reduces the survival fraction to 50%, means that both oils kill 50% of the colon carcinoma cell line [20,21]. These effects can be attributed to the bioactive compounds, linoleic and linolenic acid contents [3,33,34,36]. Phenol, linolenic acid (ω-3), palmitic and stearic acids play an important role in cancer cell inhibition [19,20]. Cytotoxicity test *in vitro* showed that the SSO and PSO oils have anticancer activity against HCT-116 cells [20,26].

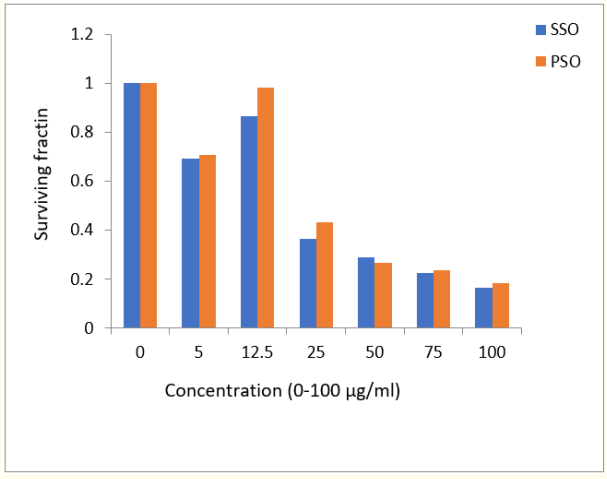

Figure 2: *Cytotoxicity effects of SSO and PSO on cancer cell line.*

Anticancer activity of SSO and PSO

Figure 3 illustrated the survival curve of HCT-116 cell which were exposed to various concentrations (5-100 µg/ml) of SSO and PSO for 48h and their growth inhibitory effect against colon cancer cell line (HCT-116). Data show SSO have a higher cytotoxic activity against HCT-116 than that of PSO (Figure 2 and 3). SSO and PSO reduced the survival fraction to 50% (kills 50% of the cancer cells) where less than 15µg of seed oil samples killed 50% of the cancer cells. These results indicated that the SSO and PSO have anticancer activities against human colon cancer cell line (HCT116) *in vitro* [15,52]. Biological activities of oils depend on their sources constituents of various origins as well as type and proportion constituents of its fatty acids [3,7,33]. Our results showed the IC_{50} value of the SSO (6.8 µg/ml) and PSO (12.2 µg/ml) were less against the HCT116 cell lines. SSO activity on human colorectal cancer cells support the evidence of the hypothesis that the high polyunsaturated fatty acids will be associated with the growth inhibition of colon cancer cell [9,15,26] reported the oil has high in behenic acid, omega-3 and omega-6 fatty acids showed anticancer effect against colon carcinoma and embryonic fibroblasts cell lines. Many investigators [20,21] showed different constituents of seed oil samples can inhibit carcinogenesis effectively and prevent the growth of colon cancer cell. Extraction method has effect on safflower and purslane seed oils production, composition and antioxidant properties [3,7,33,34].

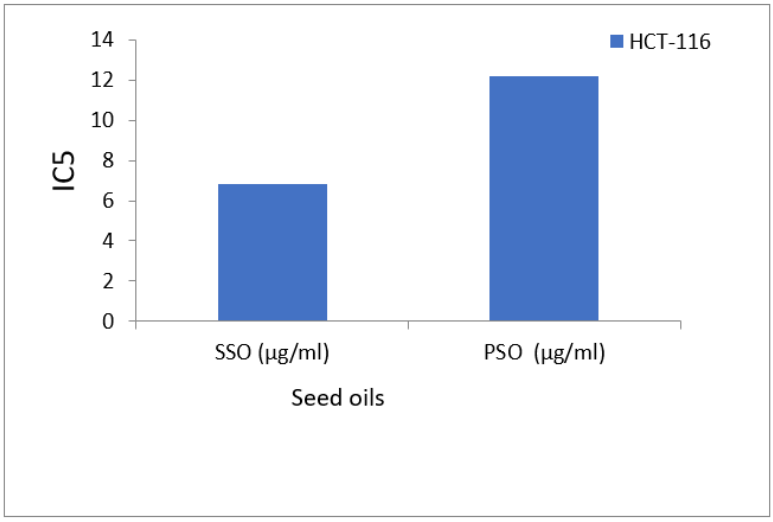

Figure 3: *IC_{50} of SSO and PSO against HCT-116 carcinoma cell line.*

In vivo studies

Safflower and purslane plants associated with people from ancient time in food and generally consumed for its nutritive values and medicinal therapeutic properties [11,12,52]. SSO and PSO inhibit cell proliferation of human colon cancer cell line (HCT-116) explained the *in vitro* anti-proliferative effects [7,26]. Anti-proliferative effects of different seed oils against different human cancer cells were reported by other investigators [9,26]. Results pointed out the SSO and PSO exhibited antioxidant and anticancer activities *in vitro*. Oral administration of SSO and PSO at a dose of 200 mg/kg did not produce any ill effects indicated the safety of SSO and PSO up to an oral dose of 200 mg/kg. Therefore, investigation of anticancer and antioxidant activities of SSO and PSO were carried out *in vivo* using rats at a dose of 200 mg/kg body weight.

Cancer induction

Carcinogenic compound commonly used is 1, 2-dimethyl-hydrazine (DMH) which is well-established for inducing colon cancer *in vivo* [31,32,37]. The colon cancer was induced by intraperitoneally injection of 1, 2-dimethyl-hydrazine (DMH) at a dose of 40 mg/kg body weight twice a week for 5 weeks [32,37]. Intraperitoneally DMH administered rats showed aberrant crypt foci initiation and adenoma development indicated the progression of colon cancer [32,37,53,54] explained the DMH was specifically targets the colon of rats. DMH-induced rat colon carcinogenesis exhibited DNA damage, pre-neoplastic lesions, oxidative stress, biotransforming enzymes, histopathological alterations and tumours [20,32,53]. Previous studies indicated that the administration periods for 8 or 12 and 16 weeks (twice/week) of intraperitoneally injection of DMH leads to the development of colon carcinoma [26,37,53]. Other study showed the administered intraperitoneally DMH weekly once for 20 weeks (20 mg/kg) leads to the development of colon carcinoma [32,52]. Rats are widely used as experimental models to study DMH-induced colon carcinogenesis [20,32,53].

Biochemical parameters

Data in table 1 showed potential effects of SSO and PSO on liver function enzymes (ALP, ALT, AST and γ-GT) in sera of carcinogenic rats (DMH) and treated rat groups. Results of total protein, albumin and globulin levels were recorded in table 1. These biochemical parameters were found to be altered in DMH-induced colon cancer control rats group [53-55]. Oxidative stress was involved in the process of tumour development of DMH carcinogenesis [54,56]. Oxidation of DNA, proteins and lipids plays an important role in common diseases, including cardiovascular, inflammatory and cancer [6,21,56]. 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 [10,20,57]. Serum transaminases are considered to be sensitive indicators of liver injury in DMH-induced colon cancer of rats where the liver was necrotized [55,58]. The hepatic damage was indicated by marked elevations in the levels of ALP, ALT, AST and γ-GT [54,55,57,58] reported the liver damage induced by chronic treatment leads to liver cell necrosis and consequently elevated levels of serum transaminases. The increases of ALP, ALT and AST levels in sera of rats were reported in cancer due to liver dysfunction [20,54,58].

Parameters	C	C/SSO	C/PSO
Total protein (g/dl)	4.02 ± 0.40	7.60 ± 0.80	6.90 ± 0.60
Albumin (g/dl)	3.10 ± 0.60	5.18 ± 0.84	5.28 ± 0.80
Globulin g/dl	0.92 ± 0.10	2.42 ± 0.20	1.60 ± 0.20
ALP (IU/L)	270.1 ± 4.14	132.20 ± 2.60	140.20 ± 2.90
ALT (U/ml)	40.60 ± 1.12	24.3 ± 1.460	28.40 ± 1.90
AST (U/ml)	52.80 ± 1.94	32.20 ± 1.80	36.94 ± 2.02
γ-GT (U/L)	162.20 ± 3.26	72.60 ± 2.18	84.02 ± 3.08

Table 1: *Biochemical parameters in sera of experimental rats (7 rats/group).*

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

C: DMH control group.

C/SSO: DMH rat group treated with SSO.

C/PSO: DMH rat group treated with PSO.

Results showed ALP, ALT and AST levels were increased significantly accompanied with significant decrease in albumin concentration in sera of DMH carcinogenic rats (C). Other investigators obtained the same results with the administration of DMH [52-54]. Significant reductions in serum total protein, albumin and globulin levels were observed in DMH-induced colon cancer rats (C) as shown in table 1.

These effects may be attributed to DMH effects of DMH inhibition of protein degradation [13,54,56]. These results are consistent to those observed by other studies [11,52]. Other investigators [9,11] reported the reduction in albumin level resulting from liver disorders and decrease in albumin synthesis due to the toxic effect of DMH leads to formation of free radicals damaging proteins [11,20,21,59]. Higher significant increases were observed in the levels of total protein, albumin and globulin in sera of rat groups administered SSO (C/SSO) and PSO (C/PSO) at dose of 200 mg/kg compared to DMH rat group C (Table 1). Seed oils administered to DMH-induced rats showed significant reductions in serum protein, albumin and globulin [13,16,27]. Higher significant increases in the levels of ALP, ALT, AST and γ-GT in sera of DMH rats (C) as previous reported [21,27,37]. Other investigators found significant increases in serum ALP, ALT, AST and γGT in liver diseases and disorders of hepatocellular damage caused by a number of agents including cancer [55-57]. These findings attributed to DMH induced colon cancer that leading to malfunction of the liver [52,54,55]. Higher decreases were observed in the levels of ALP, ALT, AST and γGT activities accompanied with significant increase in albumin concentration in rat groups treated with SSO and PSO (C/SSO and C/PSO respectively) as compared to DMH rat group (C). Results in table 1 showed significant reduction in the levels of ALP (51% and 48%), ALT (40% and 30%) and AST (39% and 30%) in sera of rat groups treated with SSO and PSO (C/SSO and C/PSO respectively) as compared to those of DMH rat group C. These results are in accordance with those reported by many investigators [54,55,57]. An increase in the ALT and AST levels in sera might be mainly due to the leakage of these enzymes from the liver into the blood stream indication the hepatotoxic effects [31,54,59]. ALP, ALT, AST are reliable markers of liver function [31,33,54,59]. Treatment of DMH carcinogenic rats with SSO and PSO reduced the activities of ALP, ALT and γ-GT in sera and consequently alleviated liver damage caused by DMH-induced colon cancer. Hepatic marker enzyme **γGT** was significant elevated in serum of rats group administered DMH rat group (C), indicating damage of the liver cell as a result of DMH carcinogenesis [54,55,57]. Higher reduction in the levels of γ-GT (55% and 42%) in sera of rat groups (C/SSO and C/PSO) given SSO and PSO respectively as compared to DMH rat group C. These results are in agreement to those obtained by other investigators [27,55]. However, the levels of **γGT** and ALT in serum of rats have been used in diagnosis of primary liver cancer [55,57,59]. Protection degree was evaluated by determining the marker enzymes (ALP, ALT, AST and γ-GT) and total proteins [27,57]. The value of ALT and AST activities in sera of rats received SSO and PSO reflected their improvement of liver function enzymes. These results are in accordance with those reported by other investigators [30,54,56].

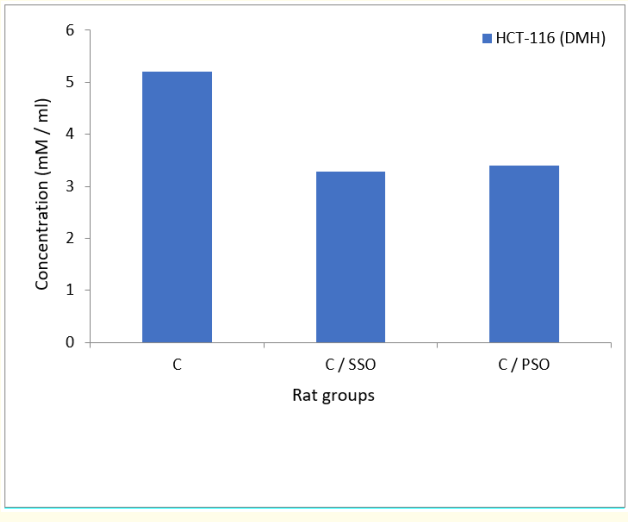

Figure 4: *Lipid peroxide levels in sera of experimental rats.*

C: DMH control group.

C/SSO: DMH rat group treated with SSO.

C/PSO: DMH rat group treated with PSO.

Lipid peroxide (LP) levels (Figure 5) were found to be higher in DMH rat (C) as reported by other investigators found DMH effect on the formation of lipid peroxidation [21,23,31]. LP levels were decreased in sera of rats received SSO and PSO as compared to those of DMH control rats (C). The highest significant decreases in the levels of LP were observed in sera of rats given SSO (C/SSO) more than those of received PSO (C/PSO) in comparison with those of DMH rats (C) as shown in figure 5. These results are in agreements with those reported by other investigators [6,31,54,57]. SSO and PSO have antioxidants scavenging free radicals and suppressed lipid peroxidation that protects cells against effect of DMH oxidative stress [31,59]. Decrease in the levels of LP activity against DMH toxicity may be due to the SSO and PSO antioxidant prevent the formation of lipid peroxidation [31,54,56,57].

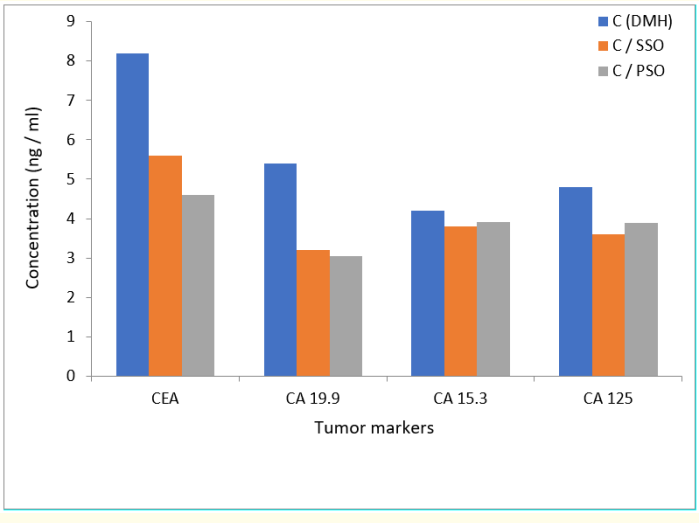

Figure 5: *CEA, CA 19.9, CA 15.3 and CA 125 levels in plasma of experimental rats.*

C: DMH control group.

C/SSO: DMH rat group treated with SSO.

C/PSO: DMH rat group treated with PSO.

CEA, CA 19-9, CA 15-3 and CA 125 were used as tumor markers for the clinical management of colon cancer [54,60]. Treated rats with SSO (C/SSO) and PSO (C/PSO) showed significant decrease in the level of CEA as compared to group C (Figure 5). Significant decreases were observed in the level of CEA, CA 19-9, CA 15-3 and CA 125 in sera of rat groups treated with SSO and PSO (C/SSO and C/PSO) as compared to those of DMH rats group C (Figure 5). CEA, CA125 and CA-19.9 have many biological aspects as adhesion, fibrosis, metastasis and apoptosis [60,61]. The reduction in the levels of CA19-9 and CA125 were observed in rat group received PSO than that received SSO. A marked reduction in the level of CEA was observed in sera of rat groups received SSO as compared to those of rat given PSO. Slightly decreases were observed in the levels of CA15-3 in sera of rat groups given SSO and PSO (Figure 5). These results are indicating the treatment role of SSO and PSO against DMH-induced colon cancer [20,37,60] used various carcinogenic or toxic materials as carbon tetrachloride and rifampicin.

Antioxidant enzymes activities

The present study was carried to investigate the antioxidant and anticancer effects of SSO and PSO against DMH-induced colon carcinogenesis using rats. GSH-T, GSH-P, GSH-R and SOD consider natural defense antioxidants scavenge free radicals and protect cells against oxidative stress. DMH rats group (C) showed different percentages of decreases in the activities of GSH-T, GSH-P, GSH-R and SOD levels in

homogenates of liver, kidney and heart tissues of the experimental rat groups (Figure 6). These findings were reported by other investigators [56,57,60]. The decreased in the activities of GSH-T, GSH-P, GSH-R and SOD could be due to the dangerous increases in the level of free radical and detoxification of toxic DMH metabolites by tumor cells and lipid peroxidation leads to inactivation of these antioxidant enzymes [31,56,57,59].

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 DMH. GSH-T activity was significant increases (Figure 6A) in plasma (51% and 35%), liver (39% and 33%), kidney (46% and 37%) and heart (44% and 26%) of rat treated with SSO and PSO (C/SSO and C/PSO). 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 [5,7,31,33,60]. Higher levels of antioxidants increase the plasma antioxidant capacity, decreasing tumor growth and inhibiting malignant cells proliferation [9,26,31,37,59].

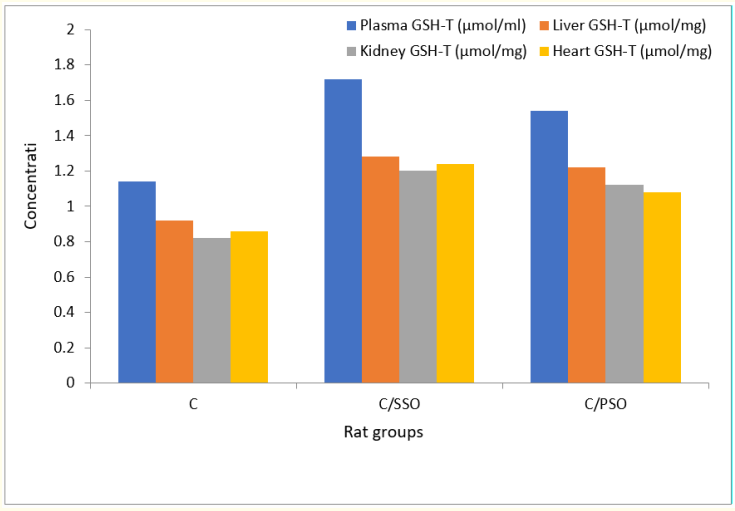

Figure 6a: *GSH-T levels in plasma, liver, kidney and heart of experimental rat groups.*

C: DMH control group.

C/SSO: DMH rat group treated with SSO.

C/PSO: DMH rat group treated with PSO.

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 damage from free radical [27,31,37]. GSH-P was significant increases (Figure 6B) in plasma (50% and 48%), liver (47% and 33%), kidney (35% and 21%) and heart (36% and 26%) of rat treated with SSO and PSO (C/SSO and C/PSO). SSO and PSO showed improve the levels of antioxidant to exert their scavenging mechanisms and exhibiting their inhibitory effects against colon carcinogenesis [5,7,20,21,29]. Similar results obtained by other investigators used different seed oils [3,9,33,57]. Other investigators [7,52,35,60] showed the SSO and PSO contains antioxidant compounds that play an important role as a protective factor for DMH-induced toxicity free radicals. GSH-P has a high potency in scavenging reactive free radicals in response to oxidative stress and detoxifies peroxides [21,29,31]. However, peroxidation inhibition by seed oils is mainly attributed to the scavenging of the reactive free radical involved

peroxidation and disturbing the antioxidant leading to oxidative stress and carcinogenesis [5,7,21,29,31]. Seed oils have polyunsaturated fatty acid and phytochemical contents might inhibits carcinogenesis effectively [29,32,33,35,56] and prevent the development of cancer *in vitro* and *in vivo* [7,26,32,35].

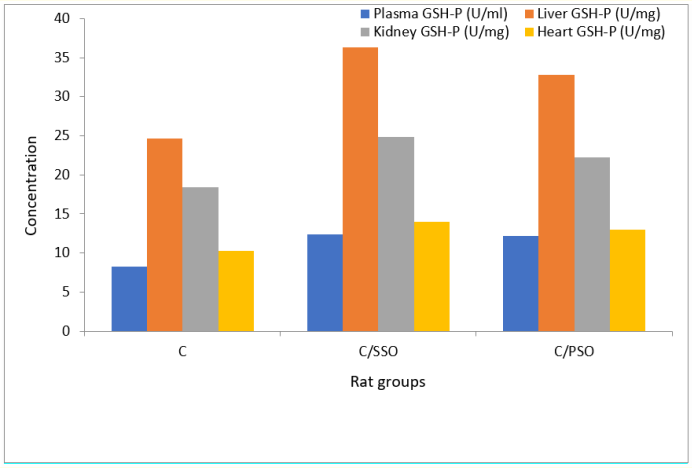

Figure 6b: *GSH-P levels in plasma, liver, kidney and heart of experimental rat groups.*

C: DMH control group.

C/SSO: DMH rat group treated with SSO.

C/PSO: DMH rat group treated with PSO.

GSH-R (Figure 6C) showed significant increases in plasma (41% and 29%), liver (61% and 54%), kidney (36% and 32%) and heart (37% and 29%) of rat treated with SSO and PSO (C/SSO and C/PSO) as compared to those of DMH rats (C). These results are in agreements with those reported by previous studies [5,7,23,57]. Other studies evidence the antioxidant properties of some natural products exhibit significant increases in GSH-P and GSH-R activities and exerted antioxidant effects [32,33,54,56]. Decrease in the levels of GSH-P and GSH-R activities during DMH toxicity might be due to antioxidant enzymes resulted during the enhanced oxidative stress and lipid peroxidation (Figure 6B and 6C). Reduction of DMH oxidative stress was observed by SSO and PSO contains fatty acids and phenolic constituents as strong antioxidants which increases GSH-P and GSH-R activities as compared to DMH rats (C). Moreover, GSH-P and GSH-R were significant increases in liver and kidney of rat groups treated with SSO and PSO (C/SSO and C/PSO) as compared to DMH-induced colon cancer rat (C). Similar observation were reported by other investigators [20,29,59] indicated the seed oils maintained liver cell and control the level of liver enzymes. GSH-P and GSH-R levels showed significant increases in liver and kidney of rat treated with SSO and PSO [5,7,21,30,56]. Free radicals are the source of lipid peroxidation derived from oxygen and SOD is the first line of defense against free radicals derived from oxygen and lipid peroxidation [31,32,35]. Results show the rats received SSO and PSO (C/SSO and C/PSO) exhibited higher SOD activity in plasma (61% and 51%), liver (60% and 56%) and kidney (37% and 32%) respectively as compared to those of rat group C (Figure 6D). These results are in agreement with those reported by several investigators [5,7,31,33,60]. SSO and PSO leads to the absence of accumulation of superoxide anion radical might be responsible for decreased lipid peroxidation in these tissues [27,31,56,59]. Other studies indicated the higher decrease in lipid peroxidation in liver and kidney of rats given seed oils being accompanied by the relatively higher increase in SOD activity [53,54,56]. Free radical scavenging and anticarcinogenic properties of SSO and PSO have been associated with their bioactive compound contents [5,7,11,29,31]. Similar results were obtained by other investigators [5,15,12,25,30,33]

finding the safflower and purslane seed oils have antioxidant and anticancer activity against colon cancer. The chief characteristics of SSO and PSO are that it is rich in polyunsaturated fatty acids, phenolic and flavonoid compounds exhibits relatively high antioxidant activity [5,7,30,33].

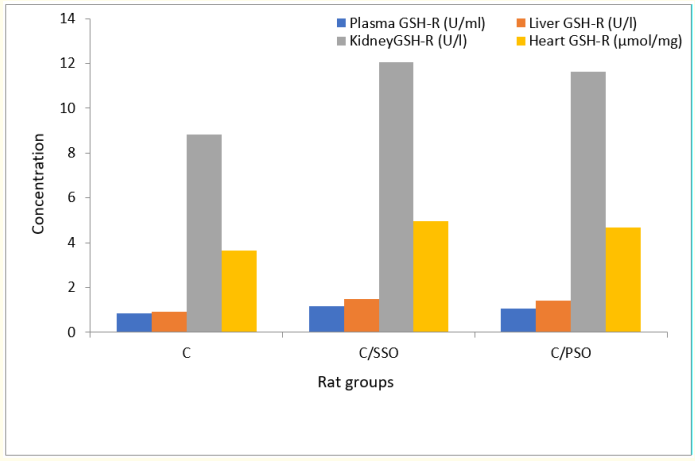

Figure 6c: *GSH-R levels in plasma, liver, kidney and heart of experimental rats.*

C: DMH control group.

C/SSO: DMH rat group treated with SSO.

C/PSO: DMH rat group treated with PSO.

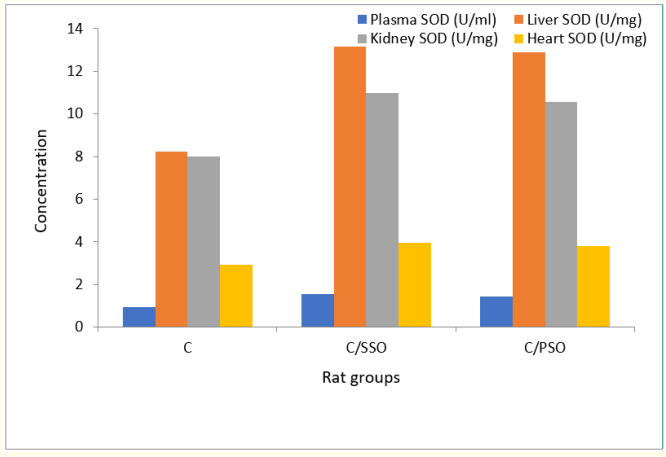

Figure 6d: *GSH-R levels in plasma, liver, kidney and heart of experimental rats.*

C: DMH control group.

C/SSO: DMH rat group treated with SSO.

C/PSO: DMH rat group treated with PSO.

From these results, it appeared that there was a positive correlation between SSO and PSO contents and SOD scavenging activity [5,7,30,33]. SSO and PSO showed inhibitory effect on hepatic enzyme activities acting as anti-lipid peroxidation agents against the permanent damage caused by DMH depending on its fatty acids and phytochemical constituents [29,31,33,35]. The most significant findings of the present study is that the SSO and PSO at the dose of 200 mg/kg body weight for 16 weeks have shown beneficial effect not only on colon cancer *in vitro* and *in vivo* but also on antioxidant activity in DMH-induced colon cancer in rats. These findings were came in harmony with other studies suggested the seed oils have ability to prevent chronic diseases related to oxidative stress including cancer [20,24,27,56,60]. However, SSO and PSO showed radical scavengers predict their antioxidant activity *in vitro* and *in vivo*. Therefore, SSO and PSO may be used as natural source of antioxidant to treat and protect the rat against oxidative stress and carcinogenic effects of DMH which improve antioxidant enzymes protect cell against oxidative stress of DMH. Safflower and purslane seed oils have been used in treated cancer, indicated to production of suitable new pharmaceutical therapeutic drugs with low cost used in low doses for treatment of different human cancer types and encourage as natural products.

Conclusion

Results suggest that the ability of safflower (SSO) and purslane (PSO) seed oils to ameliorate DMH-induced cancer is associated with its effectively regulates the antioxidant defenses, improve and elevation of some enzymes which augments the detoxification. These activities were assessed based on biochemical parameters, antioxidant enzymes level in liver, kidney and heart homogenates. Therefore, increasing oil production of seed oil crops is thus essential for more sustainable development in the future.

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