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Sea buckthorn seed oil protects against the oxidative stress produced by thermally oxidized lipids

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ABSTRACT

Thermally oxidized vegetable ghee was fed to the rabbits for 14 days with specific doses of sea buckthorn seed oil (SO). The ghee and SO were characterized for quality parameters and fatty acid composition using GC–MS. Rabbits serum lipid profile, hematology and histology were investigated. Major fatty acids were palmitic acid (44%) and oleic acid (46%) in ghee, while SO contains oleic acid (56.4%) and linoleic acid (18.7%). Results showed that oxidized vegetable ghee increases the serum total cholesterol, LDL-cholesterols, triglycerides and decrease the serum glucose. Oxidized ghee produced toxic effects in the liver and hematological parameters. Sea buckthorn oil supplementation significantly lowered the serum LDL-cholesterols, triglycerides and increased serum glucose and body weight of the animals. Sea buckthorn oil was found to reduce the toxic effects and degenerative changes in the liver and thus provides protection against the thermally oxidized lipids induced oxidative stress.

1. Introduction

Sea buckthorn (SBT) is a famous medicinal and aromatic plant, widely admired for its role to possess multiple health promoting properties. Sea buckthorn has been found to be a rich source of carotenoids, vitamin E, C, and different phenolic compounds. The seed oil had been found to help the recovery of skin injuries and skin diseases (eczema, burns, and wounds). It also helps from the damaging effects of sun on the skin, and during the radiation therapy and cosmetic laser surgery (Zeb & Khan, 2008, 2009). It had also been used in digestive ulcers and cardiovascular disease (Yang & Kallio, 2002; Zeb, 2004). The scientific studies during the recent decade confirmed the medicinal and nutritional values of sea buckthorn. The most important of these studies are the antioxidant and anti-carcinogenic properties. In animal studies, sea buckthorn products showed significant antioxidant potential. For example, Geetha et al. (2008) showed that the sea buckthorn leaf extract has a significant hepato-protective effect which was attributed to the antioxidant compounds present in the extract. Similarly, Gumustekin et al. (2010) studied the effects of nicotine-induced oxidative stress in rat heart with vitamin E and sea buckthorn extract. The authors showed that supplementation of extract increased superoxide dismutase and glutathione S-transferase activities, which may be contributing to the prevention of nicotine-induced oxidative stress in rat heart. They also suggested that the antioxidant activity of the sea buckthorn extract was due to the presence of quercetin and isorahmnetin, tocopherols, and carotenoids. Arimboor and Arumughan (2012) observed that SBT has a considerably high antioxidant and xanthine oxidase inhibitory potential shown by proanthocyanidins of the seed. Similarly, the Kim et al. (2012) findings suggested that the sea buckthorn seed extract may be a potential therapeutic agent for preventing and treating skin photo-aging. The results of Hsu, Tsai, Chen, and Lu (2009) showed that oral administration of SBT seed oil for eight - weeks significantly lowered serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), triglyceride (TG), and cholesterol, which were elevated by CCl_4 (1 mL/kg) in mice. Thus, different products of sea buckthorn have significant ameliorating properties.

Vanaspati ghee is one of the important components for the preparation of various kinds of foods. During frying or cooking the thermal stress oxidizes vanaspati ghee and produce toxic substances. These toxic substances subsequently enter the food matrix and ingested by the human (Zeb & Rahman, 2012). Experiments had shown that thermally oxidized lipids are potentially carcinogenic (Yang et al., 1998) and enhance the growth of

Abbreviations: SO, sea buckthorn oil; OG, oxidized ghee; GC–MS, gas chromatography–mass spectrometry; LDL, low density lipoproteins; HDL, high density lipoproteins; PV, peroxide value; AV, anisidine value; FFA, free fatty acids; Hb, hemoglobin; RBC, red blood cells; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin.

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hepato-carcinoma in experimental animals (Jurek et al., 2005). Recently we have shown that thermally oxidized vanaspati ghee had produced significant effects in the serum lipid profile as well as peroxidation in different tissue (Zeb & Mehmood, 2012). It is therefore necessary to find out the possible remedies to counterbalance the oxidative stress produced by oxidized lipids in our daily foods. Various plant extracts have been used to possess the antioxidant potential. The studies of Yeh, Hsieh, Lee, and Shen (2012) showed that oxidative stress induced by oxidized cholesterol can be minimized using a supplement of 0.1% of dietary Sea buckthorn. Similarly, Zeb and Hussain (2014) showed that the sea buckthorn seed powder provides protection against the toxic properties of thermally oxidized sunflower oil. The study, however does not provide information about the similar potential of the most widely used products of sea buckthorn such as seed oil. This study was therefore aimed to find out further correlation of the oxidative stress induced by thermally oxidized lipids with higher saturation and sea buckthorn oil in animal models.

2. Materials and methods

2.1. Materials

Sea buckthorn seed oil and vanaspati ghee was obtained from the commercial market. All other chemicals and reagents were of ACS grade from Sigma Aldrich (USA) or otherwise mentioned.

2.2. Thermal oxidation

Vanaspati ghee was thermally oxidized on the hot plate at 160 °C for successive 10 h in the open air to mimic the real frying conditions. After thermal oxidation the samples were stored in the refrigerator at −20 °C.

2.3. Characterization of sea buckthorn oil

The peroxide value (PV), p-anisidine value (AV) and free fatty acids (FFA) of ghee and SBT oil samples were determined using the AOCS official methods (AOCS., 1998). All samples were measured in triplicate or otherwise mentioned. Total phenolic contents (TPC) were measured using Folin–Ciocalteu reagent and quantification was carried out with the help of Gallic acid calibration curve.

Fatty acids were converted to their respective fatty acid methylesters (FAMEs). Briefly, a sample of 20 mg (±0.5 mg) was weighted in 20 mL vial. Then 6 mL of 0.5 M methanolic NaOH was added and stirred at 80 °C for 30 min. After the samples were cooled, BF_3/methanol was added and stirred further at 80 °C for 15 min. Water and n-heptane phases were separated. The heptane phase was injected into gas chromatography coupled with mass spectrometry (Agilent 5975, Agilent Germany). Fatty acids and sterol were identified from their relative and absolute retention times and also by the MS library database. The values were expressed as g/100 g determined from the peak area.

2.4. Experimental animals

All experiments were carried out according to the approved guidelines for the care and proper use of the animals of the Department of Biotechnology, University of Malakand. Rabbits selected for the study because of the highly selective and developed organ system and were easily available animals for these experiments. Rabbits were grouped by random distribution into six groups, irrespective of the gender and placed in the same animal house facility. Feed and water were provided ad libitum. The rabbits were fed with pellets from the commercial market and labeled chemical composition of the pellets showed that they contained 17.5% protein, 14.0% fiber, 2.7% fat. All animals were placed one week ahead to get familiarized with the environment.

Rabbits were classified into six groups, consisting of three animals per group. Each group is designated as the individual treatment. The rabbits were fed with oxidized ghee and sea buckthorn seed oil for 14 days, according to the feeding scheme given below: Group C: control fed on normal food; Group SO: 2 g/kg bwt SO; Group OG: 1 g/kg bwt OG; Group OGSO1: 1 g/kg bwt OG and 1 g/kg bwt SO; Group OGSO2: 1 g/kg bwt OG and 2 g/kg bwt SO; and Group OGSO3: 1 g/kg bwt OG and 3 g/kg bwt SO. The blood samples were collected in falcon tubes and centrifuge for 10 min to obtain serum for further analysis.

2.5. RSA of liver lipids

The liver tissues of each individual rabbit were taken and stored in formalin. Lipids were extracted using the modified procedure of Folch, Lees, and Sloane-Stanley (1957). The radical scavenging assays (RSA) of the lipids extracted from the tissues were measured using DPPH free radicals (Zeb & Mehmood, 2012).

2.6. Serum biochemical analysis

Serum biochemical parameters like cholesterol, HDL-cholesterol, LDL-cholesterol, ALT, and glucose were measured using HUMAN (Human Diagnostics, Germany) kits, while total triglycerides were measured using DiaSys (DiaSys Diagnostic Systems GmbH, Germany) kit.

2.7. Histopathological studies of liver

A section of the median lobe of the liver was dissected and fixed in 10% formalin buffered for at least 14 h. The section was then dehydrated with ethanol solutions and processed for embedding in paraffin. Sections of 8–10 mm in thickness were cut, deparaffinized, rehydrated, and stained. The slides were studied by binocular microscope (model M 7000 D series, Swift Instruments, Inc., Japan) and the pictures were documented using the digital camera (DCM 130) of the microscope with the resolution of 1.3 MP.

2.8. Hematological studies

For hematological examination 2 mL blood was collected from the jugular vein of rabbits in EDTA tubes. Hematology or blood profile was carried out by automatic digital machine (CELL-DYN 3200 Abbott Diagnostic Division, Canada) for the following parameters; Blood Hemoglobin Level (Hb), Blood Total RBC Level (RBC), Blood HCT Level (HCT %), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Blood Platelets Concentration, and Blood White blood cell Concentration.

2.9. Statistical analysis

All analyses were carried out in triplicate or otherwise mentioned. Data were analyzed by one-way analysis of variance (ANOVA) and Holm–Sidak method of multiple comparison method at $\alpha = 0.05$ using SigmaPlot for windows version 12.0 (Systat Software, Inc., 2011).

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