RESEARCH ARTICLE

Synergistic hepatoprotective effects of ω-3 and ω-6 fatty acids from Indian flax and sesame seed oils against CCl₄-induced oxidative stress-mediated liver damage in rats

Sunil Chikkalakshmipura Gurumallua, Tareq Aqeela, Ashwini Bhaskara, Kannan Chandramohan and Rajesha Javaraiaha,b

aDepartment of Biochemistry, Yuvaraja’s College, University of Mysore, Mysuru, India; bDepartment of Biotechnology, Yuvaraja’s College, University of Mysore, Mysuru, India; cDepartment of Zoology, Yuvaraja’s College, University of Mysore, Mysuru, India

ABSTRACT
Flaxseed (FS) and sesame seed (SS) are traditional and functional foods in traditional Indian medicine for treating various disorders. The present study investigated the hepatoprotective effects of bioactive-fatty acids (FAs) from FS and SS against carbon tetrachloride (CCl₄)-induced hepatic damage in rats. Pre and post-treatments for 28 consecutive days significantly increased the activities of in vivo antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and peroxidase (POX), whereas, lipid peroxidation (LPO) activity was markedly decreased in a dose-dependent manner in liver and kidneys. A significant reduction was observed in the hematological parameters like aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin in the serum of post-treated animals compared to the negative control. The results were confirmed histopathologically. The results suggested that the ω-3 and ω-6 FAs from flaxseed oil (FSO) and sesame seed oil (SSO), respectively, showed potential synergistic hepatoprotective and antioxidant effects that were mediated mainly by ω-3 and ω-6 FAs present in the respective seed oils.

ARTICLE HISTORY
Received 15 October 2020
Revised 17 February 2021
Accepted 17 February 2021

KEYWORDS
Flaxseed; sesame seed; polyunsaturated fatty acids; combinatorial; hepatotoxicity

CONTACT
Rajesha Javaraiah rajeshj11@rediffmail.com, rajeshaj@ycm.uni-mysore.ac.in Department of Biotechnology, Yuvaraja’s College, University of Mysore, Mysuru 570005, Karnataka, India

Supplemental data for this article can be accessed here.

© 2022 Informa UK Limited, trading as Taylor & Francis Group
1. Introduction

The liver is a vital organ that plays an important role in the detoxification of xenobiotics, drugs, viral infections, and chronic alcoholism (Hsouna et al. 2019). Carbon tetrachloride is a well-known hepatotoxicant used to induce liver damage, which involves cytochrome P450-mediated metabolic biotransformation of highly reactive metabolites, which initiate the oxidation of cellular structures and cause several diseases such as diabetes mellitus, cancer, atherosclerosis, and neurodegenerative disorders (Essawy et al. 2018, Hsouna et al. 2019). In recent years, there are upsurges in the prevalence of various degenerative and chronic diseases, including cancer, in low, middle, and high-income countries worldwide, which has led to the consumption of drugs with various disadvantages like expensive, consumption for a long time, different side effects, and interactions with other molecules, drug resistance and contraindications (Wells and Stock 2020). Consequently, the interest of consumers is being shifted toward health-promoting and disease preventive natural bioactives, which exhibit their synergistic and combinatorial potencies with each other and/or also with drugs as nutraceutical and therapeutic agents, respectively.

Flaxseed (Linum usitatissimum L.) (FS) is composed of 36–40% of oil with a high content of polyunsaturated fatty acids (PUFAs, 73% of total FA), a moderate amount of monounsaturated fatty acids (MUFA; 18%); 12–16% linoleic acid (LA, ω-6 FA) and 52–57% α-linolenic acid (ALA, ω-3 FA) and 7.97–12.30% saturated fatty acids (SFAs) of total FAs. It also contains Secoisolariciresinol diglucoside (SDG), a major lignan in addition to other nutritionally important and health beneficial components like dietary fiber, proteins, minerals, etc. (Kajla et al. 2015). Omega-3 fatty acids are extensively investigated for their triglycerides and cholesterol-lowering, high-density lipoprotein level increasing and improving endothelial function through platelet aggregation, which was reduced by antioxidant, antiatherosclerotic, anti-inflammatory, and anticarcinogenic properties (Draycott et al. 2019).

Sesame seed (Sesamum indicum L.) (SS) contains 18–25% protein and carbohydrates, 50–60% oil, which is composed of 36.4–42.1% of LA, an ω-6 FA, 16.3% of SFAs and appreciable amounts of lignans viz sesamin, sesamolin and sesaminol (Gharby et al. 2017). Linoleic acid is reported to offer protection against cancer, cardiovascular diseases, obesity, osteoporosis, immunomodulatory reactions, and oxidative stress (Basiricò et al. 2017).

Polyunsaturated fatty acids are one of the major classes, on which numerous epidemiological studies have proved that they play a pivotal role in maintaining good health. Omega-3 (ALA, C18:3) and ω-6 (LA, C18:2) FAs are the major PUFAs. Essential fatty acids (EFAs) are the precursors of long-chain fatty acids, which are converted to supremacy compounds (eicosapentaenoic acid (EPA), C20:5 and docosahexaenoic acid (DHA), C22:6) that affect many biological activities, including cell signaling, immunomodulatory, inflammation, and gene expression (Hancock et al. 2001). Currently, the growing interest of the consumers is being shifted towards a novel bioinspired strategy of cocktail two or more bioactives at a lower concentration to reduce both side and cost effects, and to enhance positive effects for the development of novel compounds by the food, pharmaceutical, and chemical industries. Synergistic and/or combinatorial effects of bioactives can be achieved if the constituents of bioactives act on different targets with each other and one another, respectively, to achieve maximal health beneficial effects by enhancing the bio-availability of one or more bioactives. A mixture of flax/sesame and flax/peanut seeds showed anti-atherogenic and hepatoprotective effects in the rats that were fed with a diet containing high fat (Makni et al. 2010). Xu et al. (2017) have demonstrated that flax oil and astaxanthin synergistically reduced the risk of oxidative stress, lipid abnormalities, cholesterol, triglycerol, hepatic steatosis, and inflammation in cardiovascular complicated cases. In our previous study, we have demonstrated the synergistic effects of flaxseed and spirulina in laying hens that resulted in significant changes in FA profile, ω-6:ω-3 ratios, ALA content, and other parameters of eggs (Rajesha et al. 2009).

We hypothesize that the combination of ALA (ω-3) and LA (ω-6) would reveal synergies that could be translated into enhanced hepatoprotective and antioxidant activities. However, no studies have reported the protective role of oil mixture of oils rich in ω-3 and ω-6 FAs used as a dietary supplement in toxicant-induced liver damage. Hence, CCl₄ was used to induce liver damage, and CCl₄ not only causes oxidative stress but also its free radicals tri-chloromethyl and tri-chloromethyl peroxyl, which are the main reactive metabolites, responsible for the toxicity of CCl₄. They interact with cellular components through the typical reactions of free radicals, fundamentally hydrogen addition and abstraction. The LPO process is an oxygen-dependent chain reaction initiated by the abstraction of hydrogen from PUFA (Recknagel 1967). Therefore, the purpose of the present investigation was to elucidate the synergistic hepatoprotective potential of FSO rich in ω-3 FA and SSO rich in ω-6 FA in CCl₄-induced oxidative stress-mediated liver damage in rats.

2. Materials and methods

2.1. Chemicals and reagents

The chemicals such as ascorbic acid, gallic acid, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), carbon tetrachloride, and standard fatty acid mixture (Supelco-37) were purchased from Sigma-Aldrich Chemical Co., St. Louis Mo, USA. Standard silymarin was procured from Micro labs, India. The diagnosis kits were purchased from Span Diagnostics Ltd., India. All other chemicals, reagents, and solvents were purchased from SD Fine-Chem Ltd., Mumbai, India.

2.2. Collection of seeds and oils extraction

Both FS and SS were procured from the Department of Oil Science, University of Agricultural Sciences (UAS), Dharwad, India. The seeds were certified as Mugad and SRI-2 varieties of FS and SS, respectively, and stored at 4°C for future analyses.
The FS and SS were subjected to screw press oil extraction (German double-barrel screws-2005, Shakti Farms, Mysuru, India) at 27 °C.

### 2.3. Fatty acid composition of FO and SO

Fixed oil compositions of the oils were determined by gas chromatography-mass spectrometry (GC-MS) analysis. The FA profile was assessed through GC-MS. Before analysis, the oils were dried under nitrogen and methylated according to the method (Morrison and Smith 1964) with boron tri-fluoride methanol complex solution (15% BF₃).

#### 2.3.1. Oil mixture preparation

The FSO and SSO were blended in the proportion of 1/5 ratio as per reports of the previous studies, and also as per recommendations of the World Health Organization (WHO) to obtain the recommended standard ω-3/ω-6 FAs ratio (Makni et al. 2010).

### 2.4. Determination of in vitro antioxidant activities

#### 2.4.1. DPPH radical scavenging activity

The free radical scavenging activities of FSO and SSO were measured by bleaching the purple-colored methanolic solution of DPPH, following the methods described by (Amiri 2010). In brief, 1 ml of DPPH (500 μM) solution was added to 100 μl of each oil sample in methanol at a 1:1 ratio and 100 mM Tris-HCl buffer (900 μl, pH 7.4). The mixture was vortexed for a minute and incubated at darkroom temperature for 20 min. The absorbance was measured at 517 nm. The scavenging effect expressed as (%) was calculated by using the equation,

\[
\% \text{ inhibition of DPPH radical} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

#### 2.4.2. Reducing power method

The reducing power of FSO and SSO was determined by the method of Yıldırım et al. (2001). In brief, 1 ml of each oil sample with a different concentration in methanol was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% (w/v) potassium ferricyanide. The mixture was incubated in a circulating water bath at 50°C for 20 min, followed by the addition of 2.5 ml of 10% (w/v) trichloroacetic acid. The reaction mixtures were centrifuged at 3000 rpm for 10 min. and the upper part of the solutions was collected, mixed with equal volumes of distilled water and a freshly prepared 0.1% (W/V) FeCl₃ solution (0.5 ml). The absorbance was measured at 700 nm.

### 2.5. Experimental design and protocol

Seventy-two male Wistar albino rats weighing 180–220 g were obtained from Invivo Biosciences, Bengaluru, after approval of the Institutional Animal Ethics Committee (Invivo/032/2018). The animals were maintained according to the principle and guidelines of the Committee for Prevention and Control of Scientific Experimentation on Animals, New Delhi. All the animals were maintained under standard laboratory conditions (24 ± 2°C, 12/12 light/dark cycle), fed with a standard pellet diet, and allowed to free access to drinking water given ad libitum during 28 days of the experimental period. Before starting the experiment, all the animals were allowed to acclimatize to laboratory conditions for a week.

The experiment was conducted in 12 groups, each consisting of 6 animals (Makni et al. 2010, Ahmed et al. 2011, Meganathan et al. 2011, Mareai et al. 2018).

- **Group-1**: Phosphate buffer saline solution (pH 7.4, 2ml/kg b.w P.O; Control)
- **Group-2**: Single intraperitoneal dose of toxicant (CCl₄, 2g/kg b.w I.P; Induction control)
- **Group-3**: Received toxicant, and after 24h they were orally supplemented with standard silymarin (25mg/kg, b.w P.O; Positive control)
- **Group-4 to 9** served as post-treated groups, received toxicant, and after 24h they were orally supplemented as follows:
  - **Group-4**: FSO [258mg per kg b.w P.O, FSO contains 150mg of ω-3 FA]
  - **Group-5**: SSO [350mg per kg b.w P.O, SSO contains 150mg of ω-6 FA]
  - **Group-6**: FSO [516mg per kg b.w P.O, FSO contains 200mg of ω-3 FA]
  - **Group-7**: SSO [700mg per kg b.w P.O, SSO contains 300mg of ω-6 FA]
  - **Group-8**: FSO + SSO [28 + 272mg/kg b.w P.O, FSO + SSO contains 25 + 125mg of ω-3 & ω-6 in 1:5 ratio]
  - **Group-9**: FSO + SSO [68 + 584mg/kg b.w P.O, FSO + SSO contains 50 + 250mg of ω-3 & ω-6 in 1:5 ratio]
  - **Group-10 to 12** served as pretreatments were orally treated as follows:
    - **Group-10**: FSO [516mg/kg b.w P.O, FSO contains 300mg of ω-3 FA]
    - **Group-11**: SSO [700mg/kg b.w P.O, SSO contains 300mg of ω-6 FA]
    - **Group-12**: FSO + SSO [86 + 584mg/kg b.w P.O, FSO + SSO contains 50 + 250mg of ω-3 & ω-6 in 1:5 ratio]

On the last day of the experimental period, these three groups were administered with the toxicant. The animal groups viz induction and positive, the post and pretreated groups (FSO, SSO, FSO + SSO) were given a single dose of toxicant (2g/kg, b.w, I.P). The animals were sacrificed under ether anesthesia and, euthanized to collect blood samples and organs for further studies.

During the experimental period, feed intake and body weight gain of the animals were measured weekly and the different organs i.e., liver, kidney, thymus, and spleen were separated and preserved for analyses.

### 2.6. Determination of in vivo antioxidant activities

Liver/kidney homogenates were prepared by using phosphate buffer of 125 mM, pH 7.4, and used to measure enzymatic and non-enzymatic antioxidants.
2.6.1. Catalase
Catalase activity was determined according to the method of (Baureder et al. 2014). In brief, 10 µl of tissue homogenates were put into 1.9 mL of 125 mM phosphate buffer of pH 7.4. The reaction was initiated by adding 1 mL of 30 mM H₂O₂. A decrease in the optical density due to the decomposition of H₂O₂ was measured at the end of 1 min at 240 nm. Catalase activity was expressed as unit per mg protein.

2.6.2. Superoxide dismutase
Superoxide dismutase activity was based on the reduction of nitroblue tetrazolium (NBT) to water-insoluble blue formazan as described by the methods of Sidduraju and Becker (2003). 5 µl of tissue homogenates were taken and 1 mL of 125 mM sodium carbonate, 0.4 mL of 24 µM NBT, and 0.2 mL of 0.1 M ethylenediaminetetraacetic acid (EDTA) were added. The reaction was initiated by adding 0.4 mL of 1 M hydroxylamine hydrochloride. Zero-time absorbance was measured at 560 nm followed by recording the absorbance after 5 min. A single unit of SOD activity was defined as the amount of enzyme required to inhibit the reduction of NBT by 50% and the activity was presented as unit per mg protein.

2.6.3. Peroxidase
The assay for POX activity was carried out by the methods of (Chidambara Murthy et al. 2002). Liver or kidney homogenate (5 µl) was taken, and to this 1 mL of 10 mM KI and 40 mM sodium acetate solutions were added. The absorbance of potassium periodide was measured at 353 nm, which indicates the amount of POX. To this mixture, 20 µl of 15 mM H₂O₂ was added, and the change in OD was recorded after 5 min. One unit of POX activity is defined as the amount of enzyme required to change 1 unit OD per min. The specific activity was expressed as unit per mg of protein.

2.6.4. Measurement of malondialdehyde formation in lipid peroxidation
Lipid peroxidation activity was carried out according to the method described by the methods of (Arora et al. 2008). Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) to form a diadduct, a pink chromogen. 5 µl of tissue homogenate and 1 mL of 0.15 M KCl were taken, and peroxidation was initiated by adding 250 µl of 0.2 mM FeCl₃ and incubated at 37 °C for 30 min. The reaction was arrested by adding 2 mL of an ice-cold mixture of 0.25 N HCl containing 15% trichloroacetic acid, 0.30% TBA, and 0.05% butylylated hydroxytoluene, heated at 80 °C for 60 min. The samples were cooled, and the results were expressed as MDA an equivalent that was calculated by using an extinction coefficient of 1.56 × 10² M⁻¹ cm⁻¹. One unit of lipid peroxidation activity is defined as the amount of TBA that gets converted to TBA reactive substances. Thus, the specific activity was expressed as unit per mg of protein.

2.7. Biochemical analysis
The blood samples were collected by direct cardiac puncture under light ether anesthesia and collected into previously labeled centrifuge tubes and allowed to clot for 30–45 min. at room temperature. The blood samples were centrifuged at 2500 rpm for 20 min and serum was collected for analyses of AST, ALT, ALP, total and direct bilirubin, total protein by using commercially available diagnostic kits were measured at 450, 570, 405, 600 and 550 nm respectively (Ferreira et al. 2010, Shaban, et al. 2014).

2.8. Organ indices
The liver, kidney, spleen, and thymus were excised from each rat and were weighed individually using a micro weighing balance and the weights were recorded (Zhang et al. 2010). The liver and kidneys were sectioned for histopathology studies. The spleen and thymus indices (Ax) were calculated using the formula.

\[ Ax = \frac{\text{weight of spleen/thymus}}{\text{body weight} \times 100} \]

2.9. Histopathological evaluation
A small portion of the right lobe of the liver drawn from the normal, induction, positive control, post, and pretreated groups was fixed in a 10% formalin solution. The tissues were processed as per standard protocol for paraffin section method and technique. Liver tissues were cut into 4 µm sections using a rotary microtome, stained with hematoxylin and eosin (Sy and Ang 2019). Further, the slides were observed under the microscope (Zeiss A2m) and photographed (Axion Vision LE64).

2.10. Statistical analysis
The data were analyzed by using the statistical package program SPSS version 16.0 (statistical package for the social sciences) by One Way Analysis of Variance (ANOVA); p < 0.05 is to determine the significant differences between treatments, and the values are expressed as mean ± SD.

3. Results
3.1. Fatty acid compositions of FSO and SSO
The FSO confirmed the presence of 5.34 ± 0.00% palmitic acid, 3.92 ± 0.01% stearic acid, and 0.09 ± 0.01% eicosanoid acid, whereas, those were in the range of 8.87 ± 0.09%, 4.90 ± 0.00%, and 0.47 ± 0.00%, respectively, in the case of SSO. Similarly, 18.05 ± 0.01% oleic acid, 12.19 ± 0.00% linoleic acid and 58.28 ± 0.01% alpha-linolenic acid were present in the FSO, while SSO exhibited their presence as 40.54 ± 0.00%, 42.86 ± 0.00%, and 0.28 ± 0.06%, respectively. Thus, the analysis showed that the sum of SFA and MUFA was higher in SSO compared to FSO. However, the sum of MUFA and PUFAs was higher in FSO compared to SSO. The degrees of unsaturation of these oils were higher than the common
vegetable oils. These levels of unsaturation could be an interesting potential in maintaining health (Makni et al. 2010, Figueiredo et al. 2017). The analyses confirmed the presence of the interested bioactives viz ALA and LA in FSO and SSO, respectively, and also met the required standards. Therefore, the oils reported in the present investigation were used in the study (Table 1).

### 3.2. In vitro antioxidant activities

In the present study, FSO and SSO were measured both alone and also in their combined form for their hydrogen donating capacity using the stable DPPH method. Free radical scavenging activity of ascorbic acid and gallic acid were from 20–100 μg, and also FSO, SSO and FSO + SSO were from 20–100 μg. The ascorbic acid and gallic acid showed activity with IC50 values 22.97 ± 0.45 and 28.94 ± 0.40, whereas, FSO, SSO, and FSO + SSO exhibited 53.98 ± 2.63, 60.23 ± 1.06, and 29.65 ± 0.33, respectively. It was observed that the scavenging activity showed by FSO + SSO was almost equal to gallic acid and near to ascorbic acid (22.94 ± 0.45). Thus, the results indicated that the activity strongly depends on the ALA and LA present in the respective oils (Figure 1(A)). It was observed that the combination of FSO + SSO, showed a noticeable scavenging activity than their alone treatments (p < 0.05). The free radical scavenging of FSO, SSO, FSO + SSO, and standard compounds were found as follows: Ascorbic acid (85.791 ± 0.423) > Gallic acid (80.162 ± 0.203) > FSO + SSO (79.145 ± 0.733) SSO (64.451 ± 1.024) > FSO (61.785 ± 1.083). All the samples showed concentration-dependent activities.

The reducing power of compounds acts as an indicator of their antioxidant potentials. The reducing powers of FSO, SSO, and FSO + SSO were compared with reference compounds for the reduction of ferric-ferrous transformation. The reducing powers of FSO, SSO, and FSO + SSO were compared with ascorbic and gallic acid at the same concentration (20–100 μg). Gallic acid showed lesser reducing capacity than ascorbic acid when they were compared in their alone treated form (Figure 1(B,C)). It was observed that the combination of FSO + SSO, exhibited a pronounced reducing power than their individual treatments (p < 0.05). The reducing power of FSO, SSO, FSO + SSO and standard compounds were found as follows: Ascorbic acid (0.856 ± 0.004) Gallic acid (0.674 ± 0.004) > FSO + SSO (0.632 ± 0.004) > FSO (0.456 ± 0.006) > SSO (0.304 ± 0.006). All the samples showed concentration-dependent activities.

#### 3.3. Organ indices, serum biochemical parameters and in vivo antioxidant activities

Hepatic injury is associated with longer exposure to hazardous chemicals, mainly from industries. Many studies have reported that reactive oxygen species (ROS) are strongly influenced by stress factors, resulting in cellular damage. These free radicals are the chief causative agents for hepatic injury (Hancock et al. 2001). In the present study In vivo synergistic/combinatorial antioxidant activity of FSO and SSO mixtures were evaluated.

Significant changes in body weight were observed in the animals of control, CCl4, silymarin, and post and pretreatment groups as presented in Table 2. Administration of CCl4 to the rats resulted in decreased body weights (16.14%) and shown a significant difference (p < 0.001) when compared to control group rats and also, increased liver and kidney weights (p < 0.001) in all the treated groups. The body weight was significantly increased (22.88%) in the silymarin treated group (p < 0.001), whereas, the liver and kidney weights were decreased (16.14%) compared to CCl4 treated rats (p < 0.001). The post-treatments of FSO (258 and 516 mg/kg b.w) and SSO (350 and 700 mg/kg b.w) gradually increased the body weights (p < 0.001) in a dose-dependent manner. The combined treatment of FSO and SSO (43 + 292 and 86 + 584 mg/kg b.w) exerted improvement in body weights (p < 0.001) along with the liver (p < 0.001) and kidney (p < 0.05) weights that were comparable with the control group. The pretreatment of FSO, SSO, and FSO + SSO (516, 700, and 86 + 584 mg/kg b.w, respectively) exhibited the highest body weight gain (p < 0.001), whereas, the liver and kidney weights were with closer values compared to the control group.

The spleen and thymus are the vital immune organs, and their indices reflect the immune functions of the body. Administration of CCl4 to rats caused significantly decreased indices (p < 0.001) of both spleen and thymus when compared to the control group. This could be due to diminished action of CCl4 treatment on the spleen and thymus of rats. The restoration of spleen and thymus indices is shown in Table 2.

The combined treatment of FSO + SSO in post and pretreatment groups showed improvement in the spleen-thymus index in a dose-dependent manner, which could be attributed to ALA and LA present in FSO and SSO oils, respectively, and were comparable to that of silymarin treated animals.

In the present study, the analysis of blood sera parameters like ASP, ALT, ALP, and bilirubin levels was carried out and the data is presented in Table 3. They were increased significantly and total protein content was decreased in CCl4 administered group when compared to the control group.

---

**Table 1. Fatty acids composition of FSO and SSO.**

| Fatty acids* | FSO (%) | SSO (%) |
|--------------|---------|---------|
| Saturated fatty acids (SFAs) |         |         |
| Lauric acid, C12:0 | 0.35 ± 0.00 | ND |
| Palmitic acid, C16:0 | 5.34 ± 0.00 | 8.87 ± 0.09 |
| Stearic acid, C18:0 | 3.92 ± 0.01 | 4.90 ± 0.00 |
| Eicosenoic acid, C20:0 | 0.09 ± 0.01 | 0.47 ± 0.00 |
| Total SFA | 9.71 ± 0.03 | 14.25 ± 0.09 |
| Monounsaturated fatty acids (MUFAs) |        |         |
| Oleic acid, C18:1(ω-9) | 12.19 ± 0.00 | 40.54 ± 0.00 |
| Gondoic acid C20:1(ω-9) | 0.08 ± 0.00 | 0.10 ± 0.01 |
| Palmitoleic acid, C16:1(ω-7) | ND | 0.11 ± 0.00 |
| Total MUF | 18.13 ± 0.00 | 43.08 ± 0.02 |
| Polyunsaturated fatty acids (PUFAs) |        |         |
| α-linolenic acid, C18:3(ω-3) | 58.28 ± 0.01 | 0.28 ± 0.06 |
| Linoleic acid, C18:2 (ω-6) | 18.05 ± 0.01 | 42.86 ± 0.00 |
| Arachidonic acid, C20:4 (ω-6) | 0.09 ± 0.00 | 0.10 ± 0.01 |
| γ-linolenic acid, 18:3(ω-6) | 0.16 ± 0.00 | ND |
| Total PUFA | 70.72 ± 0.00 | 40.92 ± 0.08 |
| Total fatty acids | 98.59 ± 0.04 | 98.26 ± 0.19 |

*Data were expresses as mean ± SD, n = 3; ND: Non detected FA.
(p < 0.001). In contrast, pretreatment of rats with silymarin helps in lowering the levels of sera parameters and restored the serum total protein. The post-treatment of rats with FSO and SSO alone (258, 350, and 516, 700 mg/kg b.w) helped to decrease blood sera parameters and to increase serum total protein levels in a dose-dependent manner when compared to the toxicant group. The co-treatment of FSO and SSO at 43 + 292 and 86 + 584 mg/kg b.w, respectively, for 28 days treated significantly controlled (p < 0.001) the elevation of blood sera parameters, which is comparable with silymarin treated group.

Pretreatment of CCl₄ intoxicated rats with FSO, SSO, and FSO + SSO (516, 700, and 86 + 584 mg/kg b.w, respectively) exhibited significant recovery in the sera parameters

Figure 1. Scavenging effects (A) and reducing ability (B,C) of standards, FSO, SSO and FSO + SSO.
### Table 2. Changes in body weight, organ weight, and spleen and thymus indices in different groups of CCl₄-treated rats.

| Treatment        | %body weight changes | Liver        | Kidney       | Spleen index | Thymus index |
|------------------|----------------------|--------------|--------------|--------------|--------------|
| PBS (Control)    | 30.52±0.11           | 1.65±0.06    | 0.12±0.31    | 0.07±0.22    |
| CCl₄             | 16.14±0.10           | 1.81±0.05    | 0.07±0.56    | 0.04±0.66    |
| Silymarin 25 mg/kg b.w | 6.78±0.11         | 1.73±0.09    | 0.11±0.51    | 0.06±0.35    |
| CCl₄+FSO 258 mg/kg b.w | 7.62±0.12       | 1.60±0.09    | 0.07±0.43    | 0.04±0.06    |
| CCl₄+SSO 350 mg/kg b.w | 7.65±0.13       | 1.69±0.12    | 0.06±0.21    | 0.04±0.33    |
| CCl₄+FSO 516 mg/kg b.w | 17.59±0.04      | 8.33±0.42    | 0.12±0.25    | 0.04±0.80    |
| CCl₄+SSO 700 mg/kg b.w | 22.88±0.07      | 11.47±0.08   | 0.25±0.07    | 0.05±0.12    |
| CCl₄+FSO+SSO (43 + 292) mg/kg b.w | 18.13±0.10  | 7.58±0.15    | 0.08±0.71    | 0.05±0.51    |
| CCl₄+FSO+SSO (86 + 584) mg/kg b.w | 7.68±0.11      | 1.73±0.09    | 0.11±0.51    | 0.06±0.35    |

### Table 3. Effects of bioactive-fatty acids rich oils from FSO and SSO on the activities of blood serum parameters of CCl₄-treated rats.

| Treatment        | Total Bilirubin mg/dL | Direct Bilirubin mg/dL | Indirect Bilirubin mg/dL | AST U/L | ALT U/L | ALP U/L | Total protein g/dL |
|------------------|-----------------------|------------------------|--------------------------|---------|---------|---------|-------------------|
| PBS (Control)    | 0.51±0.02             | 0.14±0.00              | 0.37±0.01                | 376.8±5.81 | 93±4.14 | 236.6±2.47 | 6.54±0.44         |
| CCl₄             | 2.40±0.17             | 0.91±0.03              | 1.49±0.18                | 1170.66±2.50 | 651.33±3.86 | 449.33±4.36 | 4.12±0.04         |
| Silymarin 25 mg/kg b.w | 0.58±0.02           | 0.13±0.00              | 0.45±0.01               | 264.83±4.40 | 97.57±0.64 | 190.66±4.13 | 7.02±0.05         |
| CCl₄+FSO 258 mg/kg b.w | 1.64±0.08           | 0.72±0.02              | 0.92±0.08               | 876.66±4.32 | 264.66±4.32 | 233.66±4.44 | 5.02±0.06         |
| CCl₄+SSO 350 mg/kg b.w | 1.78±0.05           | 0.73±0.02              | 1.05±0.06               | 928.66±4.32 | 281.37±4.34 | 235.37±4.34 | 4.86±0.05         |
| CCl₄+FSO 516 mg/kg b.w | 1.47±0.05           | 0.70±0.02              | 0.77±0.04               | 785.66±4.63 | 230.33±4.27 | 206.45±4.06 | 5.40±0.06         |
| CCl₄+SSO 700 mg/kg b.w | 1.65±0.06           | 0.71±0.02              | 0.93±0.04               | 897.33±4.84 | 252.33±4.63 | 216.66±4.32 | 5.06±0.06         |
| CCl₄+FSO+SSO (43 + 292) mg/kg b.w | 0.97±0.05           | 0.28±0.03              | 0.69±0.07               | 432±4.56   | 164.5±3.88 | 188.16±4.02 | 6.18±0.05         |
| CCl₄+FSO+SSO (86 + 584) mg/kg b.w | 0.71±0.06           | 0.15±0.01              | 0.56±0.07               | 312±8      | 125±4.64  | 166±6.69   | 7.54±0.09         |
| SFO 516 mg/kg b.w + CCl₄ | 1.38±0.06          | 0.62±0.02              | 0.75±0.07              | 735.66±4.27 | 218.66±4.32 | 201.16±4.83 | 5.51±0.05         |
| SSO 700 mg/kg b.w + CCl₄ | 1.43±0.06          | 0.63±0.02              | 0.79±0.07              | 863.33±4.32 | 230.83±3.37 | 204.33±4.96 | 5.12±0.09         |
| FSO + SSO (86 + 584) mg/kg b.w + CCl₄ | 0.98±0.06           | 0.25±0.02              | 0.73±0.06               | 574.33±4.45 | 171.83±2.85 | 199.5±4.37 | 6.65±0.06         |

Data were expressed as mean±SD, n=6; #Pretreatment group; *p<0.001 compared to respective CCl₄ treated group; b,p<0.05 compared to respective CCl₄ treated group; c p<0.01 compared to the respective control group; d p<0.001 compared to respective CCl₄ treated group; e p<0.001 compared to the respective control group.

(p < 0.001) compared to toxicant treated group. The treatment also showed appreciable results that were comparable among the groups with different doses of individual oils alone and also in their co-treatment. The combination of FSO and SSO exhibited significant protection (p < 0.001) against CCl₄-induced liver damage as manifested by the reduction in toxicant-mediated rise in the levels of blood sera parameters of the rats.

The significant enzymatic inhibition of CAT, SOD, and POX and non-enzymatic lipid peroxidation by bioactive FA rich oils of FSO and SSO were observed in CCl₄ intoxicated rats of the post and pretreated rats compared to the control group (Table 4). The CAT levels in liver and kidney homogenates were significantly reduced (p < 0.001) in CCl₄ treated rats when compared with control rats (Table 4). Oral administration of FSO and SSO alone in a dose-dependent manner showed appreciable improvement (p < 0.001), whereas, the combination of FSO and SSO at 43 + 292 and 86 + 584 mg/kg b.w exhibited significant improvement (p < 0.001) in CAT activity and it was dose-dependent, which are almost equivalent to silymarin and control rats of post-treated groups (Pretreatment of CCl₄ intoxicated rats with FSO (516 mg/kg b.w) and SSO (700 mg/kg b.w) alone, and also the co-treatment of FSO + SSO (86 + 584 mg/kg b.w) exerted a noticeable increase (p < 0.001) in CAT activity. Silymarin exhibited a significant (p < 0.001) increase in the activity of CAT of liver and kidney homogenates at 25 mg/kg b.w.

Superoxide dismutase is an effective antioxidant enzyme. Administration of CCl₄ causes increased production of free radicals, which reduces SOD activity. As shown in Table 4, SOD levels in both the liver and kidneys were significantly reduced (p < 0.001) in CCl₄ treated rats. Oral gavage of FSO and SSO individually treated groups exhibited a significant rise in SOD activity in a dose-dependent manner, whereas, the combined treatment of FSO and SSO at 43 + 292 and 86 + 584 mg/kg b.w showed the highest increase in the activity that is almost equal to silymarin treated and control groups in liver and kidney homogenates in a dose-dependent manner. Pretreatment with FSO and SSO alone (p < 0.01), and also in their combined form markedly elevated the activities of SOD in the liver (p < 0.001). An appreciable increase was observed in the activities of SOD of kidney homogenates of FSO (p < 0.05) and SSO alone treated groups also apart from the group of combined treatment (p < 0.01). Silymarin showed significant increase in SOD activity (p < 0.001) at 25 mg/kg b.w.
The POX levels in both the organ homogenates revealed that oral gavage of FSO and SSO alone exerted an increased effect in a dose-dependent manner, whereas, co-treatment of FSO + SSO at 43 + 292 and 86 + 584 mg/kg b.w significantly elevated the activity in dose-dependent mode (p < 0.001) (Table 4). Similarly, in pretreated groups, maximum activity was observed in the POX level of the group treated with FSO + SSO at 86 + 584 mg/kg b.w. A significant decrease (<0.001) in the activities was observed in both the organ homogenates of CCl₄ treated rats. In vice versa, POX levels were significantly increased (<0.001) in silymarin treated rats.

Administration of CCl₄ to the rats resulted in increased MDA levels that were on par with the levels of lipid peroxidation in both the homogenates (Table 4). The Post-treatment of rats with FSO (258 and 516 mg/kg b.w) and SSO (350 and 700 mg/kg b.w) significantly inhibited the formation of MDA level in liver (p < 0.01) and (p < 0.05), respectively. Similarly, the same trend was observed in the kidney homogenates of post-treatment animals with FSO (258 and 516 mg/kg b.w), SSO (350 mg/kg b.w) and SSO (700 mg/kg b.w) that showed significantly (p < 0.001), (p < 0.01) and (p < 0.001), respectively. The combination of FSO and SSO (43 + 292 and 86 + 584 mg/kg b.w) drastically inhibited the formation of MDA in the homogenates of both organs that were very close to silymarin and control groups. The pretreatment of rats with FSO, SSO, and FSO + SSO markedly inhibited the lipid peroxidation in the cell structure of the liver (p < 0.01) and kidney (p < 0.001). These findings of the present investigation provide evidence for a strong synergistic anti-oxidative potential of FSO and SSO, which is attributed to their ALA and LA, the two prominent FA, respectively, combined at a 1:5 ratio.

### 3.4. Histopathological evaluation

Figure 2 shows the representative photographs of the liver histo-architecture with Hematoxylin-eosin staining. The Histopathological examination of the liver provided supportive evidence for the mechanism of action of FSO and SSO through different biochemical parameters. Hematoxylin and eosin-stained liver tissue samples obtained from the CCl₄ (inducer) group revealed a significantly higher concentration loss of hepatic architecture, lymphocyte infiltration (shown in arrow mark), and loss of cellular boundaries when compared to the PBS (control) and post and pretreatment groups, where other cell damaged signs were observed. The FSO + SSO was similar to the control group at both concentrations.

### 3. Discussion

Carbon tetrachloride is one of the strong inducers of acute liver injury used in animal studies. It can be administered through I.P injection, oral gavage, or inhalation. The majority of researchers prefer I.P injection because of excellent reproducibility and good survival rates (Chang et al., 2005). The CCl₄ is metabolized in the liver by the cytochrome P₄₅₀, a superfamily of monoxygenases to the tri-chloromethyl radical (CCl₃). Further, this radical reacts with cellular components like nucleic acids, proteins, and lipids, resulting in the weakening of the cellular processes, which cause altered lipid metabolism (fatty degeneration and steatosis) and lowered protein quantities (Scholten et al. 2015). The cellular malfunction is evidenced by changes in hematological and biochemical parameters (Ben Hsouna et al. 2019).
To summarize, the present investigation demonstrated the synergistic hepatoprotective effects of FSO and SSO with ω-3: ω-6 PUFAs at 1:5 ratio of FA on CCl4-induced hepatic damage in rats via regulating the levels of serum biomarkers like AST, ALT, and ALP. The enzymes AST, ALT, and ALP reflect the degrees of liver injury. Hepatotoxicity or liver damage results in altered functional transition, membrane permeability, and finally leads to the leakage of enzymes into extracellular space (Vuda et al. 2012). Pre and post-treatment with FSO and SSO greatly modulated the severity of CCl4-induced hepatotoxicity. The level of enzymes returned almost to the normal levels in treated groups indicated that FSO and SSO can stabilize liver cell membranes and prevent the leakage of enzymes. Both prevention of free radical generation and their neutralization by FSO and SSO were their protection potential against liver damage, which could be other probable reasons for their healing effect (Jothy et al. 2012). Flaxseed oil also prevents the inflammation and necrotic changes in the liver by suppressing the production of inflammatory signaling molecules and reduce the inflammatory cascade by inhibiting the production of cytokines. This could be due to the presence of ω-3 FA in FSO (Latini et al. 2018).

Polyunsaturated fatty acids and their respective metabolites have been recognized for diverse metabolic functions such as maintenance of structural integrity of the cell membrane, regulation of gene expression, signaling pathways of various biochemical reactions, changes in the composition, distribution, and activities of the transcription factors, relating to absorption and metabolism of lipids, etc. (Figueiredo et al. 2017). Excessive consumption of ω-6 or a high ratio of ω-3:ω-6 PUFAs due to decreased ingestion of ω-3 FAs, promotes the pathogenesis of cardiovascular disorders, various types of cancers, inflammatory and autoimmune diseases (Simopoulos 2002, Figueiredo et al. 2017). Intake of decreased level of ω-6 FA in the diet exerts suppressive effects in the pathogenesis. The optimal ratio of ω-3:ω-6 PUFAs varies from 1:1 to 1:4, depending on the severity of chronic diseases (Simopoulos 2002). Supplementation of current Western diets with ω-3:ω-6 PUFAs at 1:1 ratio implying cardioprotective effects (Figueiredo et al. 2017). According to the American Heart Association, ω-3 FAs can modify inflammatory cascades favorably, which is an important factor in protecting anti-arrhythmic, lipid-lowering, anti-thrombotic, and other heart-related diseases. In this context, PUFAs in the diet can help to exacerbate the evolution of certain diseases, including different types of cancer (Nagy and Tiucă 2017).

As the liver is a vital organ, which is involved in most of the physiological functions, the need for hepatoprotective drugs has gradually emerged. Due to a lack of reliable drugs due to their side effects, natural bioactives have been extensively investigated for their hepatoprotective potentials (Lu et al. 2016). In this context, FSO and SSO, which are rich in bioactive FA viz ALA and LA, respectively, were used in the present investigation. As a result, the ALA and LA greatly influenced lipid metabolism that significantly ameliorated liver damage. These results are in agreement with other investigations (Makni et al. 2010).

Most of the scientific reports have claimed that EFAs from many oil seeds have the potential to counteract with free radicals mediated toxicity, which results in various tissue damages. However, only limited studies have demonstrated that PUFAs of FSO help to counteract the free radicals.

Figure 2. Microphotographs of liver histological sections (10X). A- PBS (control); B- CCl4 (induction control); C- Silymarin (positive control); D- CCl4 + FSO (258 mg/kg b.w); E- CCl4 + SSO (350 mg/kg b.w); F- CCl4 + FSO (516 mg/kg b.w); G- CCl4 + SSO (700 mg/kg b.w); H- CCl4 + FSO + SSO (43 + 292 mg/kg b.w); I- CCl4 + FSO + SSO (86 + 584 mg/kg b.w); J- #FSO (516 mg/kg b.w) + CCl4; K- #SSO (700 mg/kg b.w) + CCl4; L- #FSO + SSO (86 + 584mg/kg b.w) + CCl4; #Pre-treatment group.
mediated toxicity in animal models (Rizwan et al. 2014). Therefore, in the present investigation, we focused on assessing the hepatoprotective effects of ω-3:ω-6 PUFAs from FSO and SSO at a 1:5 ratio on toxicant-induced liver damage in rats.

The presence of fatty acid composition in the oilseeds like flax and sesame vary due to various factors, including agronomic. In FSO, ALA is a major fatty acid and it constitutes 39.90 ± 0.14% out of the total 52.24% PUFA. Similarly, in SSO, LA constitutes 28.35 ± 0.46 out of the total 28.69% PUFA (Guimarães et al. 2013). Apart from FAs, both oils also contain natural antioxidants like tocopherols and phenolic compounds, which exert different biological properties.

DPPH radical scavenging activity is the most common to determine antioxidant activity. Some studies have demonstrated that FSO and SSO significantly scavenged DPPH radicals and repaired DNA damage are attributed to their synergistic potential than the individual treatments. This method is based on a single electron transfer mechanism and measures the ability of antioxidants in oils to reduce DPPH radical (Lee et al. 2015). The –OH radical is the most reactive species that can damage DNA, proteins, and lipids. Hence, the removal of –OH is important to manage the pathogenesis of various diseases (Amiri 2010, Lee et al. 2015).

Thus, the results of our study presented in (Figure 1(A)) are in close agreement with previous reports, which are evidenced by significantly increased antioxidant activity than individual treatments against DPPH that showed a protective effect against –OH radical (Amiri 2010, Makni et al. 2010).

Reducing the power of bioactives is also a significant indicator of antioxidant activity. The compounds with reducing power show that they are electron donors, which reduce the oxidized intermediates of lipid peroxidation processes. Therefore, they can act as primary and secondary antioxidants (Hossain et al. 2012). The results of the in vitro study (Figure 1(B,C)) of the present investigation suggest that FSO + SSO, which effectively scavenged several ROS, was almost with the equal reducing capacity compared to gallic acid and near to the ascorbic acid. In this way, the results of the present study are consistent with the earlier report (Benslama and Harrar 2016).

Variation in lipid parameters such as fatty acid content, oxidative stability, iodine value, saponification value, etc. was shown to be strong risk factors for various cardiovascular diseases and fatty liver. Earlier studies have demonstrated that hypercholesterolemic rats fed with a diet rich in flax and pumpkin seed mixtures exhibited significant decrement in atherogenic index, total cholesterol, triacylglycerol, LDL/HDL ratio, increased HDL level, and improvement in antioxidant status in hepatic tissue, and renal function (Makni et al. 2008, FadlAlla et al. 2014). This could be due to the presence of ALA and LA fatty acids and fibers present in the seeds. Thus, the results of our present investigation are in line with previous reports (FadlAlla 2014).

The results of the present investigation demonstrated that the administration of CCl4 leads to an increase in organs weight, morphological changes, and pathological conditions in the liver, kidney, and other immune organs. Thus, these are in coherent with the earlier report (Peng et al. 2019). The increased levels of hepatic markers in serum are due to liver injury because these enzymes are placed in the cytoplasmic area of the cell, especially ALT, which is considered as the primary and secondary specific marker of liver injury, and are released into circulation in case of cellular damage (Vozarova et al. 2002). In the current study, serum AST, ALT, ALP, and bilirubin activities were markedly reduced in the post and pretreatment with FSO and SSO in a dose-dependent manner that largely modulated the severity of CCl4-induced liver injury (Table 3). The observed restoration of the enzymes nearly to the normal level in the treated groups was intriguing although it was less pronounced. The FSO and SSO could have involved in various metabolic processes such as stabilization of liver plasma membranes, prevention of production and neutralization of free radicals, and prevention of the leakage of enzymes. Further, it was also confirmed by the apparent alleviated histopathological damages in the liver that exhibited healing effects are in conformity with the other reports (Latini et al. 2018, Mareai et al. 2018, Wang et al. 2018).

The formation of MDA in lipid peroxidation is caused by the administration of CCl4 in a concentration-dependent manner. Lipid peroxidation is implicated in the pathogenesis of hepatic damage by free radical derivatives of CCl4 and responsible for plasma membrane damage and the release of marker enzymes of hepatotoxicity. Some of the previous reports have indicated that bioactives such as ω-3 and ω-6 FA, lignans, tannins, flavonoids, kaempferol, and other natural antioxidants can prevent lipid peroxidation and cellular damages (Makni et al. 2010, Mareai et al. 2018, Wang et al. 2018). In the present evaluation, post and pretreatments with FSO and SSO reduced the lipid peroxidation in a dose-dependent manner that could be due to the radical scavenging antioxidant property, which is attributed to their ALA and LA fatty acids, respectively (Table 4). Thus, the results of our study are in coherent with other reports.

Oxidative stress caused by free radicals is an important event in cells that can cause the aging process, inflammation, immunization, mutagenicity, and many degenerative diseases. It may result in the peroxidation of PUFAs of cell membranes and the release of excessive toxic substances such as free radicals, which circulate in the body and oxidize the low-density lipoproteins, making them potentially lethal. Many bioactives and synthetic antioxidant compounds are involved in the defense mechanism of the organisms against pathogenesis and cells from damage caused by free radicals (Amiri 2010). Endogenous antioxidants are enzymes, like CAT, SOD, glutathione peroxidase, and non-enzymatic compounds, like uric acid, bilirubin, albumin, and metallothioneins. When the endogenous factors are unable to protect the organism against the ROS, the need for additional supplementation of exogenous antioxidants like natural or pharmaceutical products, which contain active principle antioxidants like β-carotene, vitamin-C, vitamin-E, flavonoids, and other compounds arises (Makni et al. 2008).
Antioxidant enzymes are more sensitive to severe injury caused to the cells. The decrease in SOD, CAT, POX levels are the indicators of severe injury to the liver by CCl₄ and it forms free radicals during its damage. The inhibition of free radicals is important in preventing CCl₄-induced hepatotoxicity. The results of our study have revealed that rats exposed to CCl₄ along with bioactive FA rich oils showed increased SOD, CAT, and POX levels, and decreased MDA levels compared to toxicant treated group (Table 4). In this way, the findings of our current study are in close agreement with other reports (Wang et al. 2018).

The results of our present study on histopathological investigation of the liver provided complementary evidence for biochemical results. Notably, the hepatocytes of toxicant-induced rats resulted in cytophysiological changes shown by the hematoxylin and eosin staining, revealing different colors in the cytoplasm (Figure 2). This is due to the formation of highly reactive radicals associated with the oxidative threat induced by CCl₄ (Ferreira et al. 2010). The cellular injury is caused due to the accumulation of lipid hydroperoxides which causes cytotoxicity associated with peroxidation of membrane phospholipids due to lipid hydroperoxides (Halliwell and Gutteridge 2015). Thus, the findings of the present investigation confirmed that the administration of FSO and SSO alone and in their combined form FSO + SSO help to prevent biochemical and histological alterations such as loss of hepatic architecture, lymphocyte infiltration and loss of cellular boundaries, and cytophysiological changes caused by increased levels of LPO, protein oxidation and so on in CCl₄-induced oxidative stress-mediated liver damage.

4. Conclusion

Overall, the present study confirmed that the protective effects of bioactive FA rich from FSO and SSO helps in reducing oxidative stress. The in vitro studies have shown that the combination of FSO + SSO possesses the highest DPPH radical scavenging activity and reducing power than their alone form. Further, in vivo antioxidant studies have revealed that the combination of FSO + SSO significantly increased the hepatic antioxidant enzymes like CAT, SOD, POX, and decreased MDA levels in the liver and kidneys of toxicant-induced rats, which, in turn, to protect vital organs against oxidative cellular damages. Therefore, it is concluded that the co-treatment of FSO + SSO shown more synergistic antioxidant potential compared to their individual treatments. This could be due to the presence of bioactive molecules viz ω-3 (ALA) and ω-6 (LA) fatty acids in the respective oils.

The findings of our present study also reinforced the notion that combining bioactive components like ω-3 and ω-6 FA with discrete modes of action might provide a valuable approach for food, feed, and drug industries to develop health beneficial and disease preventive products rich in ω-3 and ω-6 FA with high antioxidant potential at a reduced cost. However, further research is needed in this direction to determine the role and elucidate the mechanisms regarding the combined hepatoprotective effect of ALA and LA fatty acids of FSO and SSO at the molecular level.

Acknowledgements

The authors’ sincere gratitude to Dr. Suma Mogali, Scientist, Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad, India for providing oilseed samples. The authors thank Dr. Suresh Kumar G, Senior Scientist, CSIR-Central Food Technological Research Institute, Mysuru, India for his help with GC-MS analysis and interpretation of data. Mr. SCG is grateful to the Special cell, University of Mysore, Mysuru, India for providing fellowship to carry out this research work.

Author contributions

SCG, TA, AB, KCM, and RJ conceived and designed the study. SCG, TA, and AB performed the experiments and collected the data. SCG, TA, AB, KCM and RJ analyzed and/or interpreted the data. SCG, TA, AB and KCM drafted the manuscript. SCG, TA, KCM and RJ revised the manuscript critically for important intellectual content. SCG wrote the manuscript, and all the authors have read and approved the final manuscript before its submission.

Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Sunil Chikkalakshmipura Gurumallu http://orcid.org/0000-0002-5742-5590
Tareq Aqeel http://orcid.org/0000-0001-6543-5521
Ashwini Bhaskar http://orcid.org/0000-0002-8447-6473
Kannan Chandramohan http://orcid.org/0000-0003-3801-2210
Rajesha Javaraiah http://orcid.org/0000-0002-6965-5131

References

Ahmed, E., et al., 2011. The protective effect of flaxseed oil on lead acetate-induced renal toxicity in rats. Journal of Hazardous Materials, 194, 250–255.
Amiri, H., 2010. Antioxidant activity of the essential oil and methanolic extract of Teucrium orientale (L.) subsp. tayloni (Boiss.) Rech. f. Iranian Journal of Pharmaceutical Research, 9(4), 417–423.
Arora, S., et al., 2008. Cellular responses induced by silver nanoparticles: in vitro studies. Toxicology Letters, 179(2), 93–100.
Basirico, L., et al., 2017. Comparison between conjugated linoleic acid and essential fatty acids in preventing oxidative stress in bovine mammary epithelial cells. Journal of Dairy Science, 100(3), 2299–2309.
Baureder, M., Barane, E., and Hederstedt, L., 2014. In vitro assembly of catalase. Journal of Biological Chemistry, 289(41), 28411–28420.
Ben Hsouna, A., et al., 2019. Antioxidant and hepatoprotective effect of Citrus aurantium extract against carbon tetrachloride-induced hepatotoxicity in rats and characterisation of its bioactive compounds by HPLC-MS. Archives of Physiology and Biochemistry, 125(4), 332–343.
Benslama, A., and Harrar, A., 2016. Free radicals scavenging activity and reducing power of two Algerian Sahara medicinal plants extracts. International Journal of Herbal Medicine, 4(6c), 158–161.
Chang, M.-L., et al., 2005. Comparison of murine cirrhosis models induced by hepatotoxin administration and common bile duct ligation. World Journal of Gastroenterology, 11(27), 4167–4172.
Chidambaram Murthy, K.N., Jayaprakasha, G.K., and Singh, R.P., 2002. Studies on antioxidant activity of pomegranate (Punica granatum) peel extract using in vivo models. Journal of Agricultural and Food Chemistry, 50(17), 4791–4795.
Draycott, S.A., et al., 2019. Maternal dietary ratio of linoleic acid to alpha-linolenic acid during pregnancy has sex-specific effects on placental and fetal weights in the rat. Nutrition & Metabolism, 16(1), 1.
Essawy, A.E., et al., 2018. Dual protective effect of ginger and rosemary extracts against CCl4-induced hepatotoxicity in rats. Environmental Science and Pollution Research, 25(20), 19510–19517.

FadAlia, E.A.S., et al., 2014. Hypolipidemic, antioxidant and renal protective effect of seeds mixture rich in omega-3 and omega-6 fatty acids in rats. Life Science Journal, 11(10), 866–877.

Ferreira, E.A., et al., 2010. Potent hepatoprotective effect in CCl4-induced hepatic injury in mice of phloroacetophenone from Myrcia multiflora. Libyan Journal of Medicine, 5(1), 4891

Figueiredo, P.S., et al., 2017. oxidative stability of sesame and flaxseed oils and their effects on morphometric and biochemical parameters in an animal model. Journal of the Science of Food and Agriculture, 97(10), 3359–3364.

Gharby, S., et al., 2017. Chemical characterization and oxidative stability of seeds and oil of sesame grown in Morocco. Journal of the Saudi Society of Agricultural Sciences, 16(2), 105–111.

Guimaraes, R.D.C.A., et al., 2013. Sesame and flaxseed oil: nutritional quality and effects on serum lipids and glucose in rats. Food Science and Technology, 33(1), 209–217.

Halliwell, B., and Gutteridge, J.M., 2015. Free radicals in biology and medicine. New York: Oxford University Press.

Hancock, J.T., Desikan, R., and Neill, S.J., 2001. Role of reactive oxygen species in cell signalling pathways. Biochemical Society Transactions, 29, 345–350.

Hossain, M.I., et al., 2012. Investigation of cytotoxicity and in-vitro antioxidant activity of Asparagus racemosus root extract. International Current Pharmaceutical Journal, 1(9), 250–257.

Hsouna, A.B., et al., 2019. Essential oil from halophyte Lobularia maritima: Protective effects against CCl4-induced hepatic oxidative damage in rats and inhibition of the production of proinflammatory gene expression by lipopolysaccharide-stimulated RAW 264.7 macrophages. RSC Advances, 9(83), 36758–36770.

Jothy, S.L., et al., 2012. Antioxidant activity and hepatoprotective potential of Polyalthia longifolia and Cassia spectabilis leaves against paracetamol-induced liver injury. Evidence-Based Complementary and Alternative Medicine, 2012, 561284.

Kajla, P., Sharma, A., and Sood, D.R., 2015. Flaxseed—a potential functional food source. Journal of Food Science and Technology, 52(4), 1857–1871.

Latini, J.T.P., et al., 2018. Can the indicators of chronic ethanol consumption be minimized by a continuous flaxseed intake? International Journal of Experimental Pathology, 99(5), 218–225.

Lee, A.Y., et al., 2015. Comparative study on antioxidant activity of vegetable oils under in vitro and cellular system. Journal of Agricultural Science, 73(3), 58.

Lee, G.H., et al., 2017. Protective effect of Curcuma longa L. extract on CCl4-induced acute hepatic stress. BMC Research Notes, 10(1), 77.

Lowry, O.H., et al., 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry, 193(1), 265–275.

Lu, Y., et al., 2016. Protective effect of wederolactone against CCl4-induced acute liver injury in mice. International Immunopharmacology, 34, 44–52.

Makni, M., et al., 2008. Hypolipidemic and hepatoprotective effects of flax and pumpkin seed mixture rich in ω-3 and ω-6 fatty acids in hypercholesterolemic rats. Food and Chemical Toxicology, 46(12), 3714–3720.

Makni, M., et al., 2010. Hypolipidemic and hepatoprotective seeds mixture diet rich in ω-3 and ω-6 fatty acids. Food and Chemical Toxicology, 48(8–9), 2239–2246.

Marei, S.S., et al., 2018. Protective Effect of secoisolariciresinol diglycoside in carbon tetrachloride induced hepatotoxicity in rats. Journal of Clinical & Cellular Immunology, 9(567), 2.

Meganathan, M., et al., 2011. Evaluation of hepatoprotective effect of omega 3-fatty acid against paracetamol induced liver injury in albino rats. Global Journal of Pharmacology, 5(1), 50–53.

Morrison, W.R., and Smith, L.M., 1964. Preparation of fatty acid methyl esters and dimethyleacets from lipids with boron fluoride-methanol. Journal of Lipid Research, 5(4), 600–608.

Nagy, K., and Tiuca, I.D., 2017. Importance of fatty acids in physiopathology of human body. In: A. Catala, ed. Fatty acids. IntechOpen.

Peng, C., et al., 2019. CCl4-induced liver injury was ameliorated by Qi-Ge decoction through the antioxidant pathway. Evidence-Based Complementary and Alternative Medicine, 2019, 5941263.

Rajesh, J., et al., 2009. Effects of flaxseed and spirulina biomass in layer diet on lipid profile and quality characteristics of egg yolk. Journal of Food Science and Technology, 46(6), 509–514.

Recknagel, R.O., 1967. Carbon tetrachloride hepatotoxicity. Pharmacological Reviews, 19(2), 145–208.

Rizwan, S., et al., 2014. Protective effect of dietary flaxseed oil on arsenic-induced nephrotoxicity and oxidative damage in rat kidney. Food and Chemical Toxicology, 68, 99–107.

Scholten, D., et al., 2015. The carbon tetrachloride model in mice. Laboratory Animals, 49(1), 4–11.

Shaban, N.Z., et al., 2014. Effect of Punica granatum (pomegranate) juice extract on healthy liver and hepatotoxicity induced by diethylnitrosamine and phenobarbital in male rats. Journal of Medicinal Food, 17(3), 339–349.

Sidduraju, P., and Becker, K. 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agronomic origins of drumstick tree (Moringa oleifera Lam.) leaves. Journal of Agricultural and Food Chemistry, 51(8), 2144–2155.

Simopoulos, A.P., 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomedicine & Pharmacotherapy, 56(8), 365–379.

Sy, J., and Ang, L.C., 2019. Microtomy: cutting formalin-fixed, paraffin-embedded sections. Methods of Molecular Biology, 1897, 269–278.

Vozarova, B., et al., 2002. High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. Diabetes, 51(6), 1889–1895.

Vuda, M., et al., 2012. Hepatoprotective and antioxidant activity of aqueous extract of Hybanthus enneaspermus against CCl4-induced liver injury in rats. Experimental and Toxicologic Pathology, 64(7–8), 855–859.

Wang, T.Y., et al., 2018. Protective effects of melatonin on CCl4-induced acute liver damage and testicular toxicity in rats. Indian Journal of Pharmacological Sciences, 80(6), 1100–1107.

Wells, J.C., and Stock, J.T., 2020. Life history transitions at the origins of agriculture: a model for understanding how niche construction impacts human growth, demography and health. Frontiers in Endocrinology, 11, 325.

Xu, J., et al., 2017. A combination of flaxseed oil and astaxanthin improves hepatic lipid accumulation and reduces oxidative stress in high-fat-diet fed rats. Nutrients, 9(3), 271.

Yıldırım, A., Mavi, A., and Kara, A.A., 2001. Determination of antioxidant and antimicrobial activities of Rumex crispus L. extracts. Journal of Agricultural and Food Chemistry, 49(8), 4083–4089.

Zhang, X.D., et al., 2010. Toxicologic effects of gold nanoparticles in vivo by different administration routes. International Journal of Nanomedicine, 5, 771.