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Ameliorative effect of α-tocopherol and curcumin against the deleterious effects of hydrogenated (thermally oxidized) rice bran oil in rats

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Abstract

Rice bran oil (RBO) is known for its health benefits due to its high content of natural antioxidants like tocotrienols, tocopherols, and oryzanols, which combat oxidative stress and inflammation. However, repeated heating of RBO above 110°C leads to degradation, forming harmful oxidative components and reducing its antioxidant content. This study aimed to investigate the effects of long-term consumption of thermally oxidized RBO on rats and the potential protective effects of curcumin and α-tocopherol supplementation. RBO was subjected to oxidative degradation through repeated frying cycles at temperatures ranging from 100°C to 180°C for 45 days. Physicochemical and phytochemical parameters of the oxidized RBO were analyzed. In vivo experiments evaluated serum biochemical, hematological, and histopathological parameters, along with oxidative and inflammatory responses in rats fed with oxidized RBO for 45 days. Results showed that thermal oxidation at 180°C significantly altered the chemical composition of RBO, increasing oxidative markers and reducing nutritional components. Consumption of oxidized RBO led to decreased body weight gain, elevated levels of various biochemical markers indicating liver and kidney dysfunction, hyperlipidemia, and increased oxidative and inflammatory damage in rats. However, supplementation with curcumin and α-tocopherol effectively mitigated these harmful effects. In conclusion, long-term consumption of thermally oxidized RBO negatively affects health parameters in rats, but fortification with curcumin and α-tocopherol helps alleviate these adverse effects.

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INTRODUCTION

Rice bran oil (RBO) is extracted from the outer layer of the rice grain, known as rice bran. Rice bran is removed during rice milling to produce polished white rice. Rice bran is a significant by-product of rice processing, containing abundant nutrients. Oil extraction from rice bran typically involves solvent extraction, mechanical processing, and refining processes to remove impurities [1]. Rice bran is high in vitamin E content, including phytosterols like tocotrienols, tocopherols, and oryzanols. RBO is a versatile cooking oil and has gained considerable interest for its potential health benefits. Studies have explored the unique properties of RBO, highlighting its mild flavor, high

smoke point, and good stability, making it a suitable choice for Indian cooking. RBO is stable enough to be used for frying, mainly contributing to the presence of, on average, 1.5% oryzanol. The rich profiles of antioxidants like tocotrienols and oryzanol are primarily responsible for improving lipid stability and have fascinating effects on human health, reducing oxidative stress and inflammation [2]. The oxidative stability of RBO enhances the antioxidant properties of vitamin E and oryzanol, making it healthier and more accessible to store [3]. Scientific studies have investigated the potential cholesterol-managing effect of RBO, having a positive impact on lipid profile and LDL lowering effect due to the unique composition of polyunsaturated fatty acids (PUFAs), vitamin E, sterols, and oryzanol [4]. Rice bran oil is anti-inflammatory and prevents platelet aggregation and atherosclerosis [5]. RBO can effectively lower serum cholesterol levels. RBO is considered a healthier alternative to other commonly used cooking oils. Rice bran oil contains lipolytic enzymes, mainly lipases responsible for lipolytic activity, making it unstable during storage and rancid. Separating bran by polishing rice generates frictional heat, activating lipolytic enzymes that eventually break lipids into free fatty acids (FFAs), deteriorating oil and making it rancid [6]. Repeated heating of edible oils at high temperatures during cooking is widespread. Consumption of food cooked in repeatedly heated oils poses a severe health hazard due to the formation of nutritionally undesirable products and harmful substances[7]. Heating or boiling oils is generally involved during meal preparation for cooking or deep frying in the presence of air for a long time. During this process, oil oxidation and thermal breakdown occur, and many oxidative products, such as hydroperoxide and aldehydes, are produced, which get absorbed into the fried food. The thermal oxidation of RBO during the cooking process can cause several physical and chemical changes that adversely affect the nutritional value of foods, generating FFAs and secondary oxidation products. Cooking processes, from stir-frying to deep frying, involve temperatures ranging from 100 to 200°C. Chemically refined RBO exposed to a heater at 100°C without air circulation and shielded from light showed a significant loss of α-tocopherol content after 18 days of continuous heating [8]. Zhao et al. explored the stability of RBO while cooking at 110°C and 170°C with different ratios of oryzanol to tocopherols [9]. RBO degrades above 110°C following heat exposure for more than 8 hrs, releasing oxidation products. Mishra et al. reported oxidative degradation of physically refined RBO under oven heating at 63°C and microwave heating conditions [10]. RBO, after heating at 180°C for more than 16 hrs, showed higher levels of polar components and polymer contents [11]. Consumption of thermally oxidized RBO might damage different organs due to high concentrations of oxidative products and polar compounds, although scientific exploration has not been conducted so far [12]. Heat-induced oxidation of PUFAs in RBO can induce oxidative stress in the body and cause lipid peroxidation, predominantly in the liver. Consumption of thermally oxidized sunflower [14], soybean [16], peanut, sesame, coconut [17], palm [19], soy [21] oils are reported to induce oxidative damage, liver dysfunction, fat deposition, cytotoxicity, cardiovascular diseases, atherosclerosis, cancers and neurodegenerative disorders. Long-term consumption of repeatedly heated vegetable oils is potentially harmful to liver function [22]. The antioxidant activity of RBO gets significantly reduced with an increased frying cycle [23]. To our knowledge, the biochemical damage potential of long-term consumption of thermally oxidized RBO has not been investigated. This study aims to evaluate the adverse effects of long-term thermally oxidized RBO consumption on rats. Shallow to deep frying temperature ranges (100°C and 180°C) were adopted in the study to ensure optimal oxidative degradation of RBO following repeated frying cycles. In this temperature range, chemically refined RBO high in PUFAs will likely degrade, releasing harmful components like free radicals, transfats, and malondialdehydes. The physicochemical and phytochemical parameters of oxidized RBO samples were analyzed and compared with those of fresh oil.

In vivo effects were evaluated by biochemical, hematology, growth, antioxidant enzyme, inflammatory response, and histopathological examination of rats. The current study also explored the possible role of curcumin counteracting the harmful effects of thermally oxidized RBO. This study provided crucial information about the safety aspects of consuming oxidized RBO, along with the impact of vitamin E fortifying.

MATERIALS AND METHODS

Chemicals: Tocopherol, curcumin, and rice bran oil were purchased from Ozone Pharmaceuticals Ltd., Himachal Pradesh.

Preparation of Thermally Oxidized Rice Bran Oil

Fresh RBO was stored at an average room temperature in a cool, dark place. Fresh RBO samples (200 ml) were placed into beakers and separately heated in an electric oven (Model 28, Binder GmbH, Germany) at 100°C and 180°C. Oil samples were heated for 5 hours, three times within 24 hours, to accelerate lipid oxidation and thermal degradation. The same heating pattern was followed for two more days, and thermally oxidized oil samples were collected for analysis on the third day.

Physicochemical Characterization

All the physiochemical parameters of thermally oxidized RBO 100 and RBO 180 were assessed in comparison to the normal RBO sample. The density was measured by mass over volume measurement. The oil samples' refractive index was measured using a refractometer (Mettler Toledo RM40 / RM50, Switzerland). The specific gravity was measured on the Pycnometer at 25°C following the official AOAC method. The viscosity was determined using a viscometer (Brookfield, DV-E, USA). Viscosity was determined at a constant speed of 100 rpm with a spindle number S-62, 2 (Brookfield Spindle LV, UK) and expressed in centipoise (cP). The acid, peroxide, iodine, and saponification values were determined following the standard method described in AOAC and AOCS [24].

Phytochemical Characterization

Phytosterols

The phytosterol content was analyzed using the Cercaci et al. procedure with slight modifications [25]. One milliliter of oil sample was mixed with 4 mL of potassium hydroxide ethanol solution (2 mol/L) and shaken in a heated water bath at 90°C for 1 hour for saponification. After cooling, 5 mL of n-hexane and 1 mL of deionized water were added at room temperature. This mixture was shaken vigorously to extract the unsaponifiable matter. The supernatant was extracted thrice with hexane and pooled using the same procedure.

Total Phenols

The liquid-liquid extraction method was applied to extract the phenolic fraction of oil samples by phase-wise vortexing and phase separation. In the first phase, the oil sample was treated with a methanolic hexane solution (1:1), followed by hexane-ethyl acetate (85:15), and finally eluted with methanol. The solvent was removed under vacuum at 35°C. The Folin-Ciocalteu reagent was used to measure the total phenolic content spectrophotometrically at 765 nm and expressed as mg of GAE (gallic acid equivalents) per 100 gm oil [26].

β-Carotene

The procedure described by Gimeno et al. was followed with some modifications to isolate and quantify β-carotene [27]. Following the extraction of unsaponifiable matter, the organic phase obtained was evaporated using a rotary evaporator (Rotavapor R-300, Fisher Scientific, USA) at 40°C. The residue was evaporated under a stream of nitrogen and redissolved in methanol. Quantification was performed immediately to avoid the oxidation and decomposition of the β-carotene. The compositions of beta-carotene in rice bran oil samples were analyzed using a double-beam UV-Vis Spectrophotometer (SL160, India). The β-carotene content of the oil samples was expressed as mg/100 gm [28].

Tocopherols

The qualitative estimation of tocopherols was performed with a high-performance liquid chromatography (HPLC) system (Agilent Technologies 1260, USA) consisting of a 515 pump, a UV-visible detector, a Thermo C 18 (250 × 4.6 mm) 5 µm column, and the Data Ace software. The mobile phase consisted of water-methanol-acetonitrile (90:5:5 v/v) at a flow rate of 1 ml/min. The phenolic residue was dissolved in a methanol and water (1:1) mixture, sonicated for 25 min, and filtered through a 0.45 µm filter. The stock solution was further diluted sufficiently with methanol to get a sample solution of 10 µg/mL. The sample volume injected was 20 µl, and the retention time of tocopherols was detected at 290 nm [29].

In-Vivo Experimentation

The effects of α-tocopherol and curcumin treatment against hydrogenated rice bran oil consumption-induced damage were performed on

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rats. The study comprised ten animals, 5 per group for biochemical parameters and 5 for carrageenan-induced inflammation. Oils and drugs were given to each group of animals orally for 45 days, and they were provided with food, water, and libitum throughout the experimental period. Wistar albino rats of both sexes weighing between 150–200 g maintained under standard laboratory conditions at 22±2°C, relative humidity 50±15%, and photoperiod (12 hrs dark and light) were used for the experiment. Commercial pellet diet (Agro Corporation Private Limited, Bangalore, India) and water were provided ad libitum. The experiments were approved by the Institutional Animal Ethics Committee (CPCSEA/IAEC/09/2022/47), and experiments were performed following the Committee for Control and Supervision of Experiments on Animals (CCSEA), New Delhi guidelines.

Body Weight

Each animal was weighed individually on day zero before starting the experiment. Body weights were taken once a week and on the 45th day of the experiment as the final body weight. Body weight gain (in percentage) was calculated with respect to the initial body weight.

Relative Organ Weight

The relative weight of the heart, liver, and kidney was estimated based on the following formula: Relative organ weight (%) = Weight of organ/Body weight of rat × 100.

Serum Biochemical Parameters

On the 46th day, animals were sacrificed under anesthesia after overnight fasting. Animals were quickly dissected. The tissues of interest, the liver, kidney, and heart, were rapidly isolated, placed in cold saline, and weighed. Blood was collected by terminal cardiac puncture in test tubes without anticoagulant for serum separation.

Total cholesterol, triglyceride, HDL, SGOT, SGPT, glucose, albumin, blood urea nitrogen, creatinine, and bilirubin were estimated in serum. Total cholesterol and triglyceride level were assessed using enzymatic assays (cholesterol CHOD-PAP, Biolabo, France, and triglyceride GPO-PAP, Elabscience, USA), SGOT, SGPT were determined by the method of Reitman and Frankel [30]. Albumin was determined using a Biuret kit (Elabscience, USA), and HDL was measured by enzymatic colorimetric assays (HDL-C plus 3rd generation colorimetric assay kit, Elabscience, USA). Glucose was estimated using a glucometer (Accu Chek Instant Glucometer, Roche Diabetes Care, India). Blood urea nitrogen was estimated according to the method of Patton and Remp [31]. Creatinine was estimated by Jaffe's reaction and first described by Jaffe in 1886 [32]. The orange color produced by creatinine when it reacts with picric acid in an alkaline medium was measured at 520 nm. Bilirubin was estimated using an alkaline azobilirubin method kit, Nescauto BIL-V3 (Nippon Shoij Kaisha, Ltd., Osaka, Japan).

Hematology

Total WBC was counted using a Neubauer hemocytometer (Feinoptik, Germany), and ESR was measured following the standard pathological method described by Ghai [33]. The Arsenazo 3 colorimetric method was employed to estimate serum calcium (Atlas Medical, Germany) [34]. The ammonium molybdate technique was used for measuring serum phosphorus, and the Calmagite technique for estimating serum magnesium [35].

Oxidative Parameters of Liver

Oxidative enzyme levels were assessed on liver homogenate. The weighted section of the liver was homogenized in ice-cold 0.25 M sucrose, centrifuged at 3000 rpm for 10 min, and the supernatant was kept for estimation of lipid peroxidase, SOD, GSH, catalase, vitamin E, and total protein. Thiobarbituric acid reacts with malondialdehyde (MDA), a secondary product of lipid peroxidation. The developed color is reddish-pink, which is estimated at 532 nm. The level of lipid peroxidases was expressed as nmol of MDA per gm of liver protein [36]. Inhibition of nitroblue tetrazolium (NBT) reduction was the basis for SOD activity measurement following the technique of Kakkar et al [37]. One enzymatic unit of SOD corresponds to the amount of protein present in the form of enzyme in the sample required to inhibit the reduction of NBT by 50% and is expressed as unit/mg of protein. Estimation of glutathione peroxidase activity was performed following the method described by Ellman [38], and the results were expressed as nmol/mg of liver protein. Catalase was assayed colorimetrically at 620 nm and expressed as a unit of H_2O_2 consumed/min/mg protein described by Sinha [39]. Thiol radical or the sulfhydryl group in

glutathione forms a colored complex with 5-5 Dithiobis-2 nitrobenzoic acid (DTNB), measured colorimetrically at 412 nm. Vitamin E was estimated using the method of Jargar et al. [40][40]. A lipid extract of homogenate, 0.5 ml, was added with 1.5 ml of ethanol and 2.0 ml of petroleum ether and centrifuged. The supernatant was evaporated to dryness below 80°C, and 0.2 ml of 2-2' dipyridyl solution (0.2%) and ferric chloride (0.5%) were added, kept in the dark for 5 minutes. Butanol 4 ml was added, and the color developed was spectroscopically estimated at 520 nm.

Serum Inflammatory Parameters

Serum Interleukin 6 (IL-6) and Tumour Necrosis Factor-alpha (TNF-α) concentrations were determined using a commercially available high-sensitivity indirect sandwich enzyme-linked immunosorbent assay (Bender MedSystems, Austria) [41].

Inflammatory Response

Five rats per group were used to assess the inflammatory response on the 18th and 45th day of treatment. Inflammation was induced by 0.1 ml of Freund's Complete Adjuvant (FCA) subcutaneous injection in the subplanter region of the right hind paw on the 18th and 45th day of treatment. An equal volume of saline injected into the left paw served as the control [42]. Paw volume was observed on the 19th and 46th day using a mercury Plethysmometer (Classic Scientific, Mumbai).

RESULTS

Physicochemical and Phytochemical Properties of Thermally Oxidized RBO

The thermal oxidation of RBO with periodically repeated heating for three days accelerated thermal degradation and changed the oil from a golden yellow color to burnt amber (Figure 1). The process of thermal oxidation at 180°C had a noticeable impact on the chemical composition of the RBO, resulting in a significant increase in density, refractive index, and viscosity ($P < 0.05$-0.001) and a decrease in specific gravity ($P < 0.001$) (Figure 2). Thermal degradation at 180°C resulted in significantly increased acid value, saponification value, and peroxide value, while the iodine value was decreased ($P < 0.05$-0.001) (Figure 3). The thermal degradation of RBO at 100°C and 180°C led to extremely significant decreases ($P < 0.001$) in phytosterol content but no loss of total phenols. Oxidation of RBO at 100°C and 180°C showed respective losses of 28% and 62% in β-carotene content (Table 1). The HPLC chromatogram showed the qualitative presence of tocopherols in the normal and thermally oxidized rice bran oil samples (Figure 4).

Figure 1 Normal and thermally oxidized rice bran oil at 100 and 180°C for 3 days

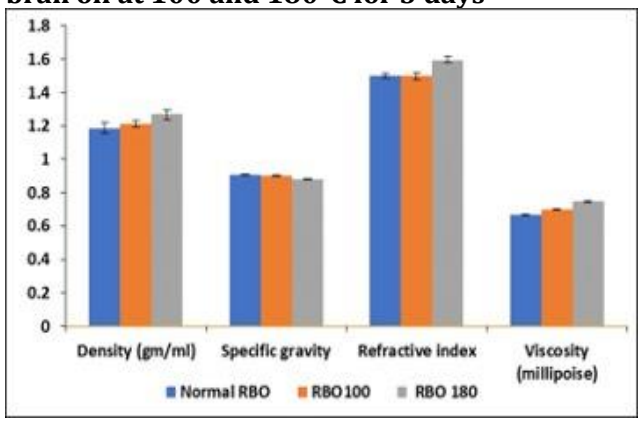

Figure 2 Physical characteristics of thermally oxidized rice bran oil.

Each value is a mean of three determinations. RBO = Rice bran oil, RBO 100 = Rice bran oil oxidized at 100°C, and RBO 180 = Rice bran oil oxidized at 180°C.

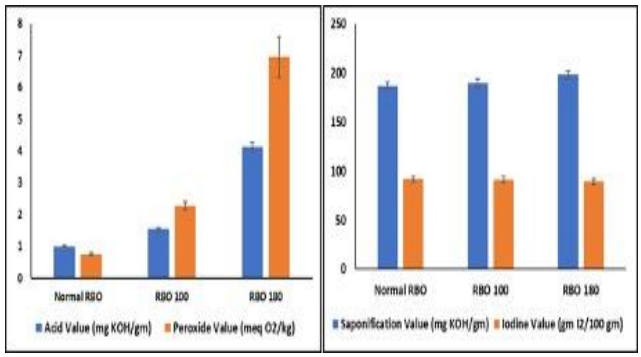

Figure 3 Chemical characteristics of thermally oxidized rice bran oil.

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Each value is a mean of three determinations. RBO = Rice bran oil, RBO 100 = Rice bran oil oxidized at 100°C, and RBO 180 = Rice bran oil oxidized at 180°C.

Table 1 Phytochemical characteristics of thermally oxidized rice bran oil

Categories	Phytosterol (mg/100 gm)	Total phenols content (mg GAE/100)	β-carotene content (µg/gm)
Normal RBO	187.89 ± 4.24	2.73 ± 0.73	0.108 ± 0.002
RBO 100	155.24 ± 3.45***	2.14 ± 0.77ns	0.077 ± 0.006***
RBO 180	143.11 ± 3.12***	1.87 ± 0.58ns	0.041 ± 0.004***

Each value is a mean of three determinations. RBO = Rice bran oil, RBO 100 = Rice bran oil oxidized at 100°C, and RBO 180 = Rice bran oil oxidized at 180°C.

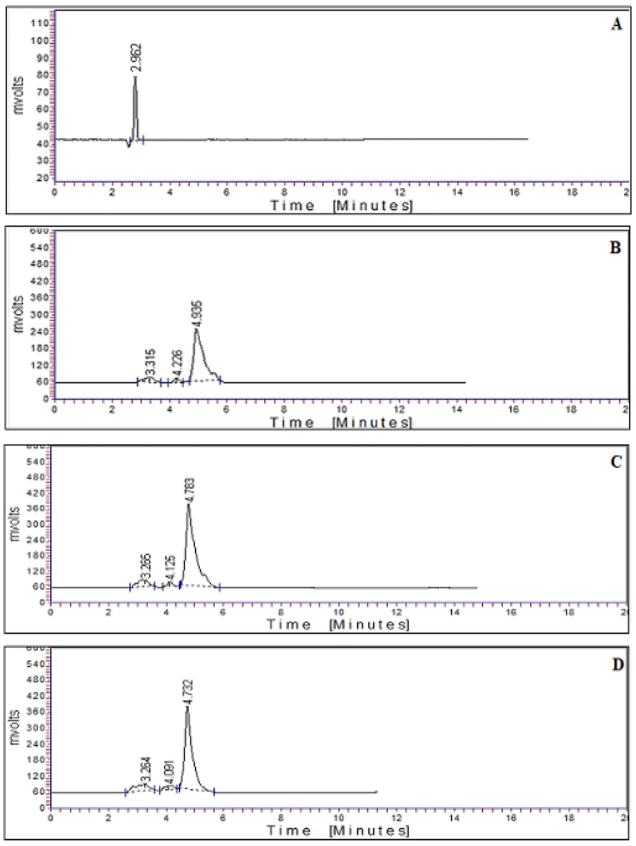

Figure 4 HPLC retention time of tocopherols in the normal and thermally oxidized rice bran oil sample.

A: 10 ppm standard α-tocopherols 2.962 min; B: Normal rice bran oil 3.315 min; C: Thermally oxidized rice bran oil at 100°C 3.265 min; D: Thermally oxidized rice bran oil at 180°C 3.264 min

Effect of Long-Term Thermally Oxidized RBO Consumption on Rats

Body Weight and Relative Organ Weight

Consumption of oxidized BRO for 45 days showed a reduction in body weight gain concerning zero-day weight compared to the control group. Concurrent consumption of curcumin and α-tocopherol alone and oxidized RBO also showed a similar tendency (Figure 5). Thermally degraded RBO consumption did not significantly affect the relative weight of the heart, whereas α-tocopherol increased heart weight ($P < 0.05$). The kidney weight of rats treated with RBO oxidized at 180°C decreased significantly ($P < 0.05$), which was reversed by curcumin 100 mg/kg co-treatment. Heating RBO at both 100°C and 180°C increased relative liver weight ($P < 0.05$-0.01). Conversely, curcumin and α-tocopherol normalized it significantly (Table 2).

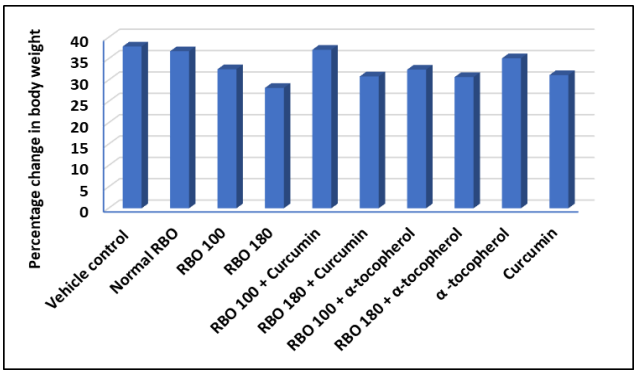

Figure 5 Effect of daily oral administration of thermally oxidized rice bran oil up to 45 days on body weight of rats.

All the values are Mean ± SEM of six values (n = 6). The percentage change in body weight on 45th day was calculated compared to the respective zero day body weight of each group.

Hematological Parameters

Normal and thermally degraded RBO consumption has a non-significant effect on plasma calcium and phosphorus levels, but thermally degraded RBO significantly ($P < 0.001$) enhances total WBC count. Normal RBO, as well as curcumin and α-tocopherol treatment for 45 days, significantly increases ($P <$

Table 2 Effect of daily oral administration of thermally oxidized rice bran oil up to 45 days on relative organ weight of rats

Treatment groups	Dose	Heart (gm/100 gm B.W)	Liver (gm/100 gm B.W)	Kidneys (gm/100 gm B.W)
Vehicle control (Normal Saline)	1.5 ml	0.72 ± 0.014	2.64 ± 0.03	1.42 ± 0.08
Normal RBO	1.5 ml	0.80 ± 0.026^{ns}	2.81 ± 0.09	1.38 ± 0.03^{ns}
RBO 100	1.5 ml	0.81 ± 0.035^{ns}	3.12 ± 0.03*	1.33 ± 0.04^{ns}
RBO 180	1.5 ml	0.75 ± 0.026^{ns}	3.80 ± 0.09**	1.23 ± 0.06*
RBO 100 + Curcumin	1.5 ml + 100 mg/kg	0.68 ± 0.044^{ns}	2.94 ± 0.02*	1.42 ± 0.09^{ns}
RBO 180 + Curcumin	1.5 ml + 100 mg/kg	0.73 ± 0.027^{ns,ns}	3.16 ± 0.08^{**,a}	1.56 ± 0.06^{ns,a}
RBO 100 + α-tocopherol	1.5 ml+ 300 mg/kg	0.77 ± 0.055^{ns}	3.12 ± 0.04*	1.28 ± 0.02^{ns}
RBO 180 + α-tocopherol	1.5 ml + 300 mg/kg	0.69 ± 0.071^{ns,ns}	3.39 ± 0.05^{*,a}	1.49 ± 0.06^{ns,ns}
α -tocopherol	300 mg/kg	0.86 ± 0.062*	2.72 ± 0.03^{ns}	1.55 ± 0.09^{ns}
Curcumin	100 mg/kg	0.77 ± 0.034^{ns}	2.80 ± 0.05^{ns}	1.67 ± 0.05**

All the values are Mean ± SEM of six values (n = 6). *P<0.05, **P<0.01, ***P<0.001, and ns = not significant when compared to the respective control group. ^{a}P<0.05 when compared to the RBO 180 group. B.W. = Body weight.

Table 3 Effect of daily oral administration of thermally oxidized rice bran oil up to 45 days on hematological parameters of rats

Treatment groups	Dose	WBC (10^3/mm3)	Calcium (mg/dl)	Magnesium (mg/dl)	Phosphorus (mg/dl)
Vehicle control	1.5 ml	6.52 ± 0.27	4.32 ± 0.56	1.35 ± 0.05	4.92 ± 0.73
Normal RBO	1.5 ml	7.28 ± 0.64^{ns}	5.65 ± 0.14^{ns}	2.06 ± 0.08**	5.30 ± 0.82^{ns}
RBO 100	1.5 ml	10.06 ± 1.43***	4.19 ± 0.22^{ns}	1.95 ± 0.09**	4.28 ± 0.55^{ns}
RBO 180	1.5 ml	10.38 ± 1.14***	4.43 ± 0.58^{ns}	1.85 ± 0.07*	4.03 ± 0.64^{ns}
RBO 100 + Curcumin	1.5 ml + 100 mg/kg	7.45 ± 0.87^{ns}	4.31 ± 0.55^{ns}	1.96 ± 0.02**	4.35 ± 0.56^{ns}
RBO 180 + Curcumin	1.5 ml + 100 mg/kg	7.43 ± 0.81^{ns}	4.20 ± 0.36^{ns}	1.56 ± 0.04*	4.14 ± 0.66^{ns}
RBO 100 + α-tocopherol	1.5 ml+ 300 mg/kg	8.50 ± 0.75^{ns}	5.28 ± 0.50^{ns}	1.77 ± 0.05*	3.86 ± 0.37^{ns}
RBO 180 + α-tocopherol	1.5 ml + 300 mg/kg	8.50 ± 0.81^{ns}	5.79 ± 0.43^{ns}	1.86 ± 0.06*	4.75 ± 0.91^{ns}
α -tocopherol	300 mg/kg	8.36 ± 0.62^{ns}	5.48 ± 0.95^{ns}	2.38 ± 0.08**	5.11 ± 0.72^{ns}
Curcumin	100 mg/kg	7.28 ± 0.38^{ns}	5.45 ± 0.83^{ns}	2.86 ± 0.09***	4.64 ± 0.33^{ns}

All the values are Mean ± SEM of six values (n = 6). *P<0.05, **P<0.01, ***P<0.001, and ns = not significant when compared to the respective control group.

0.01-0.001) plasma magnesium levels, while thermal degradation reduces RBO's ability to improve plasma magnesium levels, which remains unaffected even after curcumin and α-tocopherol co-treatment (Table 3).

Liver Biochemical Parameters

Consumption of normal RBO for 45 days has significantly (P < 0.05) decreased total cholesterol and triglyceride levels and increased HDL content in rat plasma. Treatment with 180°C heated oil significantly (P < 0.05-0.01) increased total cholesterol, triglyceride, SGOT, and SGPT levels and decreased HDL levels. Curcumin and α-

Table 4 Effect of daily oral administration of thermally oxidized rice bran oil up to 45 days on liver biochemical parameters of rats

Treatment groups	Dose	TC (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	SGOT (U/L)	SGPT (U/L)
Vehicle control	1.5 ml	53.28 ± 2.70	74.41 ± 3.50	40.15 ± 1.80	111.03 ± 4.20	130.10 ± 3.89
Normal RBO	1.5 ml	45.12 ± 2.44*	62.29 ± 4.33*	53.13 ± 1.95*	104.03 ± 5.10^{ns}	122.93 ± 3.77^{ns}
RBO 100	1.5 ml	59.26 ± 3.21^{ns}	76.79 ± 4.36^{ns}	38.38 ± 1.33^{ns}	159.90 ± 5.68*	146.17 ± 3.72*
RBO 180	1.5 ml	92.55 ± 4.40**	119.17 ± 4.52**	31.51 ± 1.20*	225.03 ± 5.08**	152.07 ± 3.81*
RBO 100 + Curcumin	1.5 ml + 100 mg/kg	56.74 ± 3.81^{ns}	71.50 ± 3.61^{ns}	42.85 ± 1.82^{ns}	123.60 ± 4.12^{ns}	137.73 ± 3.29^{ns}
RBO 180 + Curcumin	1.5 ml + 100 mg/kg	69.25 ± 4.03^{*,b}	94.11 ± 3.09^{*,b}	37.08 ± 1.49^{ns,a}	162.07 ± 4.32^{*,b}	140.77 ± 3.47^{ns,a}
RBO 100 + α-tocopherol	1.5 ml+ 300 mg/kg	51.92 ± 3.88^{ns}	78.48 ± 3.75^{ns}	36.12 ± 1.23^{ns}	154.43 ± 4.82*	143.47 ± 4.10^{ns}
RBO 180 + α-tocopherol	1.5 ml + 300 mg/kg	66.82 ± 3.05^{*,b}	88.05 ± 4.22^{*,b}	37.14 ± 1.45^{ns,a}	185.17 ± 4.02^{*,b}	146.27 ± 3.22^{ns,a}
α -tocopherol	300 mg/kg	55.13 ± 2.47^{ns}	75.46 ± 3.40^{ns}	46.47 ± 1.38*	109.94 ± 4.83^{ns}	132.17 ± 4.03^{ns}
Curcumin	100 mg/kg	52.30 ± 2.84^{ns}	71.43 ± 3.61^{ns}	40.54 ±1.35^{ns}	94.41 ± 2.88*	125.07 ± 3.14^{ns}

All the values are Mean ± SEM of six values (n = 6). *P<0.05, **P<0.01, ***P<0.001, and ns = not significant when compared to the respective control group. ^{a}P<0.05 and ^{b}P<0.01 when compared to the RBO 180 group.

tocopherol co-treatment potentially (P < 0.05-0.01) reversed these effects towards normality (Table 4).

Kidney Biochemical Parameters

Normal RBO and curcumin treatment for 45 days significantly reduced blood sugar levels (P < 0.05).

Thermally oxidized RBO did not affect blood sugar and albumin levels, whereas curcumin elevated (P < 0.05) albumin levels. Consumption of 180°C oxidized RBO for 45 days significantly (P < 0.05-0.01) increased serum BUN, creatinine, and bilirubin content. Curcumin and α-tocopherol co-treatment efficiently (P < 0.05-0.01) normalized these parameters towards normality (Table 5).

Anti-oxidative and Anti-inflammatory Effects

Normal RBO has significantly (P < 0.05) increased vitamin E, SOD, and catalase levels. Oxidized RBO consumption for 45 days has a non-significant effect on vitamin E and glutathione peroxidase levels of liver tissue. Normal RBO, α-tocopherol co-administration, and α-tocopherol alone have significantly (P < 0.05-0.01) increased plasma vitamin E content and reduced lipid peroxidation. RBO oxidized at 180°C has evidently (P < 0.01-0.001) increased liver lipid peroxidase and decreased SOD and catalase levels following 45 days of treatment. Curcumin and α-tocopherol co-treatment effectively (P < 0.05-0.001) reversed the oxidative damage by normalizing the levels of oxidative enzymes (Table 6).

Normal RBO has significantly (P < 0.05) lowered TNF-α and IL6 levels compared to the control group animals. Animals fed with hydrogenated RBO heated at 180°C for 45 days showed profoundly high (P < 0.01) ESR, TNF-α, and IL6 levels compared to the control group. Simultaneous administration of curcumin and α-tocopherol has significantly (P < 0.05-0.01) reversed the serum ESR, TNF-α, and IL6 levels (Table 7). Feeding rats with normal RBO, curcumin, and α-tocopherol for 45 days showed a profound anti-inflammatory response, respectively 11.06%, 17.45%, and 21.38% reduction in FCA rechallenge-induced paw thickness. Feeding of oxidized RBO at 180°C has enhanced the inflammatory reaction

Table 5 Effect of daily oral administration of thermally oxidized rice bran oil up to 45 days on kidney biochemical parameters of rats

Treatment groups	Dose	Glucose (mg/dl)	Albumin (gm/dl)	BUN (mg/dl)	Creatinine (mg/dl)	Bilirubin (mg/dl)
Vehicle control	1.5 ml	96.75 ± 2.66	3.38 ± 0.24	23.16 ± 1.20	0.42 ± 0.004	0.38 ± 0.003
Normal RBO	1.5 ml	82.36 ± 2.01*	4.40 ± 0.71^{ns}	21.45 ± 1.05^{ns}	0.46 ± 0.007^{ns}	0.31 ± 0.002^{ns}
RBO 100	1.5 ml	92.97 ± 2.17^{ns}	3.70 ± 0.45^{ns}	25.62 ± 1.67^{ns}	0.73 ± 0.002*	0.52 ± 0.008*
RBO 180	1.5 ml	101.41 ± 3.04^{ns}	3.20 ± 0.74^{ns}	29.50 ± 1.70*	0.81 ± 0.008**	0.59 ± 0.009*
RBO 100 + Curcumin	1.5 ml + 100 mg/kg	92.62 ± 2.33^{ns}	3.29 ± 0.90^{ns}	22.42 ± 1.64^{ns}	0.55 ± 0.006*	0.40 ± 0.003^{ns}
RBO 180 + Curcumin	1.5 ml + 100 mg/kg	90.80 ± 2.80^{ns}	3.52 ± 0.40^{ns}	24.38 ± 1.55^{ns,a}	0.64 ± 0.005^{*,b}	0.41 ± 0.005^{ns,a}
RBO 100 + α-tocopherol	1.5 ml+ 300 mg/kg	95.44 ± 3.65^{ns}	3.77 ± 0.33^{ns}	24.77 ± 1.06^{ns}	0.62 ± 0.004*	0.43 ± 0.006^{ns}
RBO 180 + α-tocopherol	1.5 ml + 300 mg/kg	98.95 ± 3.06^{ns}	3.25 ± 0.17^{ns}	23.60 ± 1.55^{ns,a}	0.71 ± 0.006^{**,a}	0.45 ± 0.007^{ns,a}
α -tocopherol	300 mg/kg	94.52 ± 3.22^{ns}	4.39 ± 0.92^{ns}	22.51 ± 1.76^{ns}	0.47 ± 0.007^{ns}	0.36 ± 0.005^{ns}
Curcumin	100 mg/kg	84.33 ±3.65*	5.44 ± 0.47*	21.64 ± 1.65^{ns}	0.56 ± 0.009^{ns}	0.34 ± 0.006^{ns}

All the values are Mean ± SEM of six values (n = 6). *P<0.05, **P<0.01, ***P<0.001, and ns = not significant when compared to the respective control group. ^{a}P<0.05 when compared to the RBO 180 group.

towards the FCA challenge with a 35.92% increase in paw thickness. Simultaneous administration of curcumin and α-tocopherol has reduced the inflammatory response towards the FCA challenge (Figure 6).

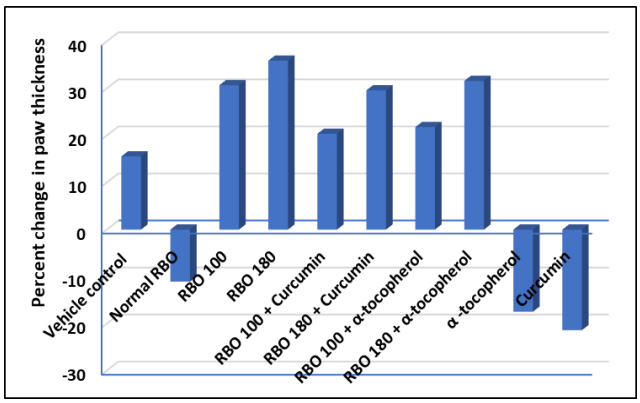

Figure 6 Effect of daily oral administration of thermally oxidized rice bran oil up to 45 days on paw inflammation of rats.

All the values are Mean ± SEM of six values (n = 6). The percentage change in paw thickness on 45th day was calculated compared to the respective 19th day value for each group.

DISCUSSION

This study investigates the physicochemical and phytochemical alterations of RBO subjected to high-temperature thermal oxidation and the consequent long-term consumption-associated biochemical effects on rats. Thermal oxidation of RBO by repeated heating at 180°C significantly alters physicochemical and phytochemical parameters, adversely affecting the nutritional value. Long-term consumption of thermally oxidized RBO induced liver and kidney dysfunction, hyperlipidemia, oxidative damage, and inflammatory disorders in rats. Long-term consumption of repeatedly heated RBO at a deep-frying temperature range is potentially harmful to the liver and kidneys, along with other associated health risks. Fortification with curcumin and α-tocopherol can effectively counteract the harmful effects of thermally oxidized RBO.

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Table 6 Effect of daily oral administration of thermally oxidized rice bran oil up to 45 days on oxidative parameters of rats

Treatment groups	Dose	Vit E (µmol/mg)	SOD (U/mg)	Catalase (U/mg)	GSH (nmol/mg)	Lipid peroxidase (nmol/mg)
Vehicle control	1.5 ml	2.05 ± 0.05	4.47 ± 0.14	32.25 ± 1.22	2.25 ± 0.95	5.52 ± 0.66
Normal RBO	1.5 ml	2.42 ± 0.03*	5.24 ± 0.42*	38.23 ± 1.57*	2.27 ± 0.26^{ns}	5.41 ± 0.60^{ns}
RBO 100	1.5 ml	1.86 ± 0.05^{ns}	2.26 ± 0.56**	25.24 ± 1.05*	1.36 ± 0.57^{ns}	8.57 ± 0.46**
RBO 180	1.5 ml	1.07 ± 0.08^{ns}	1.28 ± 0.95***	22.46 ± 1.60**	1.35 ± 0.94^{ns}	10.60 ± 0.73***
RBO 100 + Curcumin	1.5 ml + 100 mg/kg	2.04 ± 0.04^{ns}	3.14 ± 0.56^{ns}	30.36 ± 2.11^{ns}	2.43 ± 0.18^{ns}	7.52 ± 0.49*
RBO 180 + Curcumin	1.5 ml + 100 mg/kg	1.97 ± 0.03^{ns,a}	2.22 ± 0.84^{**,b}	27.37 ± 2.04^{ns,a}	1.95 ± 0.73^{ns}	7.53 ± 0.78^{*,b}
RBO 100 + α-tocopherol	1.5 ml+ 300 mg/kg	2.27 ± 0.06**	3.51 ± 0.35^{ns}	31.69 ± 1.38^{ns}	2.46 ± 0.62^{ns}	7.51 ± 0.54*
RBO 180 + α-tocopherol	1.5 ml + 300 mg/kg	2.88 ± 0.08^{**,b}	3.18 ± 0.65^{ns,c}	29.90 ± 1.05^{ns,a}	1.73 ± 0.44^{ns}	8.50 ± 0.28^{**,a}
α -tocopherol	300 mg/kg	2.68 ± 0.05*	5.89 ± 0.37*	33.66 ± 1.44 ^{ns}	2.52 ± 0.63^{ns}	4.55 ± 0.71*
Curcumin	100 mg/kg	1.25 ± 0.06^{ns}	6.24 ± 0.75*	39.22 ± 1.28*	2.50 ± 0.56^{ns}	5.02 ± 0.80^{ns}

All values are expressed concerning liver protein. All the values are Mean ± SEM of six values (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001, and ns = not significant when compared to the respective control group. . aP < 0.05, bP < 0.01, and cP < 0.01 when compared to the RBO 180 group. Vit E = vitamin E, SOD = superoxide dismutase, GSH = glutathione peroxidase.

The natural antioxidant profile of RBO, including α-tocopherol, γ-tocotrienol, and γ-oryzanol, protects lipids from oxidation. The rich bioactive antioxidant profile of RBO protects lipids from thermal oxidation, but bioactive compounds can degrade during repeated deep frying in high temperatures. The unsaturated fatty acid content of RBO is very high, usually up to 75%, which gets degraded first, acting as a substrate for oxidation and saving the bioactive antioxidants[13]. Tocopherols and tocotrienols in oils protect against oxidation and degradation upon exposure to high temperatures by oxidizing themselves to quinones and dimers. α-Tocopherol in RBO remained almost unaffected upon deep-frying cycles, whereas γ-tocotrienol degrades considerably during deep frying [23]. On the contrary, Bruscatto et al. reported a reduction of 28.65% α-tocopherol content in RBO submitted to prolonged heating at 100°C, which can be nearly 100% at 180°C temperature [8]. Among the vitamin E analogs, γ-tocotrienol is less stable and quickly oxidizes during deep oil frying [43]. γ-Oryzanol is one of the most potent naturally occurring antioxidants. γ-Oryzanol degradation in RBO is almost parallel to that of γ-tocotrienol with the number of frying cycles due to oxidation [44]. It is crucial to evaluate the pathological effects of consuming oxidized RBO by repeated deep frying, as food absorbs oil of the same composition left in the frying pan during frying.

RBO contains considerable high PUFA (high linoleic acid), MUFA (high oleic acid), and a small amount of saturated fatty acid (SFA) [45]. Thermal

Table 7 Effect of daily oral administration of thermally oxidized rice bran oil up to 45 days on inflammatory parameters of rats

Treatment groups	Dose	ESR (mm/hr)	TNFα (pg/ml)	IL6 (pg/ml)
Vehicle control	1.5 ml	5.26 ± 0.75	1.61 ± 0.08	41.07 ± 1.56
Normal RBO	1.5 ml	4.51 ± 0.82^{ns}	1.08 ± 0.06*	32.55 ± 1.93*
RBO 100	1.5 ml	8.08 ± 0.69**	3.22 ± 0.13**	52.51 ± 2.33^{ns}
RBO 180	1.5 ml	8.25 ± 0.94**	4.44 ± 0.42***	75.46 ± 2.68*
RBO 100 + Curcumin	1.5 ml + 100 mg/kg	5.55 ± 0.55^{ns}	1.93 ± 0.08^{ns}	43.49 ± 1.55^{ns}
RBO 180 + Curcumin	1.5 ml + 100 mg/kg	6.27 ± 0.68^{ns,b}	2.16 ± 0.03^{ns,b}	57.80 ± 2.11^{*,a}
RBO 100 + α-tocopherol	1.5 ml+ 300 mg/kg	6.08 ± 0.46^{ns}	2.23 ± 0.02*	52.36 ± 2.06^{ns}
RBO 180 + α-tocopherol	1.5 ml + 300 mg/kg	7.51 ± 0.82^{*,a}	2.38 ± 0.04^{*,b}	55.26 ± 2.52^{*,a}
α -tocopherol	300 mg/kg	5.22 ± 0.64^{ns}	1.44 ± 0.06^{ns}	46.25 ± 2.30^{ns}
Curcumin	100 mg/kg	4.14 ± 0.22^{ns}	1.29 ± 0.05^{ns}	36.45 ± 1.76^{ns}

All the values are Mean ± SEM of six values (n = 6). *P<0.05, **P<0.01, ***P<0.001, and ns = not significant when compared to the respective control group. ^{a}P<0.05 and ^{b}P<0.01 when compared to the RBO 180 group. ESR = erythrocyte sedimentation factor, TNFα = tumor necrosis factor alpha, IL6 = interleukin 6.

oxidation of RBO by periodically repeated heating for three days degrades the oil, as evidenced by a significant increase in density, refractive index, and viscosity with a decrease in specific gravity, suggesting an alteration in molecular composition. Chemical degradation aligns with increased acid value, saponification value, peroxide value, and decreased iodine value, corroborating oxidative deterioration of RBO at elevated temperatures [46]. The decrease in the iodine value of RBO following prolonged high-temperature treatment was observed by Dymińska et al [45]. Results agree with previous reports of increased peroxide value and viscosity of RBO following repeated deep frying cycles [13]. A decrease in the iodine value suggests a reduction in the degree of unsaturation to an increase in saturation during oxidation [47]. These changes indicate thermal degradation, producing undesirable polymerized triglycerides and oxidized fatty acids [48]. The findings suggest that repeated heating at 180°C caused about a 62% loss of the total β-carotene content of RBO, whereas, at 100°C, the loss of carotene was less significant. β-Carotene in oil undergoes isomerization as a dominating reaction when heated above 140°C during the initial 2 to 4 hrs [49]. The observed loss of phytosterol and β-carotene following thermal degradation of RBO signifies the susceptibility of these bioactive compounds to thermal stress. Tocopherol in the degraded oil samples indicates resilience to the oxidation process and aligns with their known antioxidant properties [50].

Rats consuming RBO for 45 days showed a nominal decrease in body weight gain, in agreement with the previous report by Yang et al [51]. The substantially reduced body weight gain of rats consuming oxidized RBO may be due to decreased fat absorption and increased fecal fat excretion. Feeding of thermally degraded oil has significantly lowered retroperitoneal tissue weights and lipid contents [52]. Thermally oxidized oil consumption is associated with reduced body weight gain [15][53]. Decreased kidney weight and increased liver weight of rats fed with oxidized RBO indicate oxidative stress over these organs. Curcumin and α-tocopherol partially mitigate the deleterious effects, indicative of a potential defensive role against oxidative damage [55]. Rats showed a significant increase in WBC count after consuming thermally degraded RBO, which implies an immune response to oxidative damage induced by oxidized oil. Rice bran and RBO are reported to have a rich magnesium content [57]. Normal RBO increased plasma magnesium level, whereas oxidized RBO feeding did the reverse. Altered plasma magnesium levels associated with long-term consumption of oxidized RBO indicate a complex interplay between oxidized oil and mineral homeostasis.

Consumption of normal RBO for 45 days resulted in decreased plasma levels of cholesterol, triglyceride, and glucose and an increase in HDL. RBO has significantly elevated vitamin E, SOD, and catalase, whereas it inhibited the secretion of the pro-inflammatory cytokines IL-6 and TNF-α levels.

The reduction in paw thickness upon rechallenge with FCA in animals treated with normal RBO further supports its protective role against oxidative damage. The results presented here are in line with previous reports on RBO consumption. The practical hypolipidemic effect of RBO is attributed to the presence of γ-oryzanol and tocotrienols [59]. A study on hyperlipidemic subjects fed with RBO in cooked meals for four weeks showed decreased lipid parameters, improved antioxidant status, and inflammatory markers after the intervention [60]. RBO improved the plasma lipid profile of type 2 diabetic patients consumed daily for five weeks [61]. Sixty days of feeding of RBO on rats has efficiently reduced pro-inflammatory cytokine release from macrophages [62]. A high-fat diet in rats tends to upregulate lipid-regulatory genes, sterol regulatory element-binding protein-1 (Srebf1), and peroxisome proliferator-activated receptors-α (Ppara) inducing lipogenesis. The associated insulin resistance caused oxidative stress downregulating catalase (CAT) and superoxide dismutase (SOD1) genes. RBO supplementation improves insulin resistance by downregulating lipogenic genes and balancing oxidative stress [63]. RBO exerts an anti-inflammatory response via modulating mitochondrial respiration of macrophages [5].

Oxidized RBO has induced dysfunction in plasma lipid and kidney profile, underlining its ability to affect liver and kidney function. Blood levels of cholesterol, triglyceride, SGOT, SGPT, BUN, creatinine, and bilirubin were elevated in RBO 180°C treated animals with decreased HDL content. RBO has high thermal stability and is considered healthier among various cooking oils. RBO degrades above 110°C, forming oxidative products and polar and polymeric components [8]. This study is the first to explore the deleterious effects of hydrogenated RBO long-term consumption. Thermally oxidized sunflower, soybean, peanut, sesame, coconut, palm, and soy oils are associated with oxidative damage, liver dysfunction, fat deposition, cytotoxicity, cardiovascular diseases, atherosclerosis, cancers, and neurodegenerative disorders [14]. In vivo, deep-fried oil can affect plasma and mitochondrial membranes [64]. The alterations in antioxidant enzyme levels in liver tissues and the inflammatory markers in the serum highlight the oxidative stress induced by consuming oxidized RBO. Hydrogenated RBO has aggressively enhanced paw thickness upon FCA rechallenge, indicating a potential inflammatory response due to oxidative damage.

An in-depth understanding of the protective mechanisms involved in the protective role of curcumin and α-tocopherol against chronic stress has prompted their supplementation for attenuating hydrogenated RBO-induced damages. Near normalization of liver and kidney biochemical profiles after co-administration of curcumin and α-tocopherol justifies their protective role in ameliorating oxidative stress-related metabolic disturbance. Curcumin is the active constituent of turmeric, having well-established antioxidant and anti-inflammatory properties that potentially reduce oxidative stress [65]. Curcumin supplementation reduced oxidative stress markers, chronic inflammatory response, and improved lipid profile. The ability of curcumin and α-tocopherol to reverse antioxidant enzyme levels and pro-inflammatory markers emphasizes their antioxidant and anti-inflammatory properties. α-Tocopherol is one of the most potent naturally occurring scavengers of reactive oxygen and reactive nitrogen species. Dietary BRO has a rich presence of α-tocopherol ranging between 50-300 mg/kg [8][66]. α-Tocopherol is an isoform of vitamin E that inhibits the radical chain propagation within lipid domains by converting to an oxidized product, α-tocopherol free radical [67]. Prolonged high-temperature heating is associated with the loss of antioxidant bioactive components of RBO [8][23]. Dietary supplementation of α-tocopherol can provide additional shielding against loss of content. α-Tocopherol supplementation reduced IL-6 and TNF-α levels in rats exposed to chronic stress [68].

Conclusion:

RBO is rich in tocotrienols, tocopherols, oryzanol, and omega-3 fatty acids, conferring many health benefits [57]. The study results substantiate the rich presence of phytosterol, total phenol, β-carotene, and α-tocopherols in RBO. The potential beneficial effects of normal RBO observed in this study include weight reduction, hypoglycemia, increased plasma magnesium, hypolipidemia, antioxidants, and anti-inflammatory properties, which corroborate previous reports. RBO has been previously reported to attenuate obesity, hyperlipidemia, and inflammation in mice and rats.

However, there is a lack of data regarding chronic consumption-associated detrimental effects of hydrogenated RBO.

The results of the current study suggest that hydrogenated RBO can potentially cause an imbalance in lipid metabolism, dysregulation of kidney function, induce oxidative stress and immune response, and lead to chronic inflammation. Further research is needed to explore the molecular mechanisms and related signaling pathways to validate these observations and elucidate the precise interaction between RBO oxidative products and biological systems.

Supplementation of curcumin and α-tocopherol can potentially protect against these adverse outcomes, emphasizing the importance of incorporating antioxidant-rich supplements in the diet to counteract the adverse effects of oxidized edible oils.

Ethical Approval

No ethical approval was necessary for this study.

Author Contribution

All authors made substantial contributions to the conception, design, acquisition, analysis, or interpretation of data for the work. They were involved in drafting the manuscript or revising it critically for important intellectual content. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work, ensuring its accuracy and integrity.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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