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Research Article

Fabrication of Nutraceutical Beverage from Saffron (*Crocus sativus* L.) Extract and Studying Its Health Effects

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A saffron extract-based beverage (SEBB) was formulated and characterized based on its sensory attributes and health benefits. The main bioactive compounds of saffron extract (crocin and safranal) were quantified. Three formulations of SEBB were prepared based on the sucrose concentration: SEBB 1 contained 65 g of sucrose per 500 ml, SEBB 2 contained 17.5 g, and SEBB 3 contained 79.5 g. The SEBB most desired by consumers was then subjected to biochemical analysis to evaluate its antioxidative effects on the damage induced by food contaminated with carbon tetrachloride (CCl_4). Fifteen albino rats were split into five groups and treated with different doses of CCl_4 or SEBB according to the planned animal experiment for 62 days. Sensory evaluation illustrated that SEBB 1 had the highest acceptability scores. The content of crocin and safranal was 23.039 and 4.135 ppm, respectively. The SEBB ameliorated the increased activity of enzymes involved in liver and kidney function and improved the total antioxidant capacity, blood glucose, and lipid profile.

1. Introduction

Foods often become contaminated by carbon tetrachloride (CCl_4) when they are fumigated with it; exposure from contaminated drinking water can also occur as a result of inhalation of CCl_4 that has volatilized during showering or other uses of domestic water, such as clothes washing [1]. CCl_4 is an organic compound which appears as a colorless and volatile liquid [2]. Its toxicity usually follows the inhalation of the vapor in a poorly ventilated environment or the ingestion of contaminated food (especially grains) or groundwater and hence causes severe health problems to humans and animals [3, 4]. Subsequent damage caused by CCl_4 has been linked to many mechanisms, including the formation of reactive oxygen species (ROS) and the disruption of redox equilibrium. Inflammation and the induction of programmed cell death have also been described [5].

Saffron is a rare spice that is produced from the perennial blooming plant *Crocus sativus* L., a member of the family Iridaceae [6]. Crocin and safranal are the major active compounds in saffron [7]. There are many water-soluble carotenoids including crocin [8]. Saffron has been found in scientific research to have therapeutic efficacy with a variety of pharmacological actions, including antioxidant [9, 10] and cardioprotective effects [11]. Saffron's ability to reduce the damage caused by genotoxic substances has been proven in subsequent investigations utilizing a variety of tests [12]. These protective properties have been related to a reduction in oxidative stress caused by saffron [13].

Nutraceutical beverages can be an important part of the human diet since they can contain key bioactive chemicals, commonly found in the form of pills, capsules, liquids, and other medications [14], that can help treat and prevent chronic illness [15], as well as providing needed hydration. However, the taste and flavor quality of such beverages is

the most important factor for adoption [16]. As a result, the formulation of high-quality beverages with good flavor, scent, and shelf-life stability is critical for achieving the appropriate levels of consumption required for health promotion [17] and disease prevention, as well as contributing to the treatment and prevention of chronic diseases [18, 19].

Despite the large number of antioxidant studies on phytochemical constituents conducted around the world, a review of published articles revealed that there is a need to investigate the effect of a nutraceutical beverage made from saffron extract against CCl_4-induced damage to bridge the gap between studies in recent years, especially since saffron has traditionally been used for folk medicine or flavoring. The goal of this study was to see if saffron extract-based beverages could protect rats from CCl_4-induced harm.

2. Materials and Methods

2.1. Chemicals and Reagents. The dried stigmas of the saffron flower, sugar, were obtained from a local market in Erbil, Iraq. Crocin, safranal, citric acid, and gum Arabic were obtained from Sigma (St. Louis, MO, USA). All solvents were of analytical grade and were obtained from Scharlab S.L. (Spain). Sandwich ELISA kits were purchased from (Elabscience, USA).

2.2. Preparation of Saffron Extract. The extraction of saffron was conducted according to the method provided by Koul and Abraham [20]. Briefly, 50 g of stigma powder was macerated in 500 ml of ethanol for 24 h before being shaken for 4 h with a magnetic stirrer. After that, the solution was filtered using the Whatman filter paper. The resulting clear solution was concentrated by evaporation under vacuum to dryness at temperatures below 40°C using a rotary evaporator (PER FIT, Indian origin). The supernatant was then collected and frozen for 24 h.

2.3. Quantification of Crocin and Safranal in Saffron Extract. Quantification of safranal and crocin in the saffron extract was performed according to the modified method of Mradu et al. [21]. The sample was prepared by dissolving the saffron extract in ethanol at a concentration of 1000 μg/ml, and then, 20 μl of the sample was injected into a high-performance liquid chromatograph (HPLC; model Sykam, Germany) through a C18 column (250 × 4.6 mm I.D., particle size 5 μm; Agilent Technologies, USA). The crocin was detected at 320 nm and safranal at 440 nm at 25°C, and the flow rate was 1 ml/min for crocin and 0.8 ml/min for safranal. The mobile phase for crocin consisted of 40 : 60 ethanol : water (% v : v) and for safranal was 80 : 20 ethanol : water (% v : v) which was filtered using a 0.45 mm filter.

2.4. Preparation of Nutraceutical Beverage from Saffron Extract. The whole experimental scheme is shown in Figure 1. The nutraceutical saffron beverage was prepared as reported by [22, 23] with some modifications. Briefly, sucrose was added to 500 ml of water at three concentrations of 65, 17.5, and 79.5 g, with fixed amounts of 0.5 g of citric acid and 0.75 g of gum Arabic; the suspension was stirred with a domestic shaker (medium velocity). The prepared

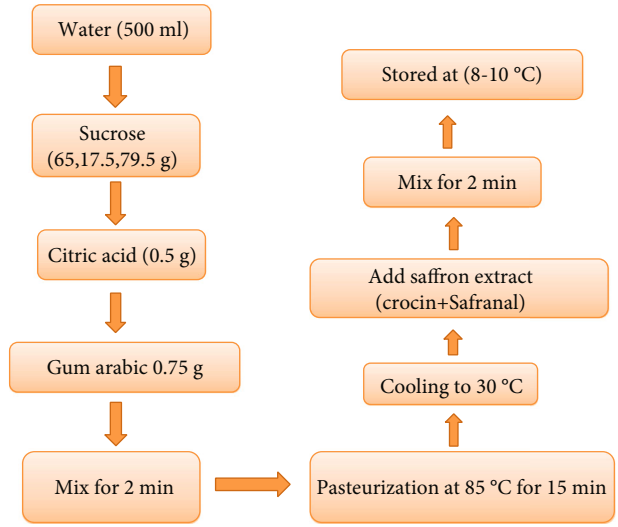

Figure 1: Flowchart of the nutraceutical beverage production with saffron extract.

beverages were heated to 85°C for 15 min and allowed to cool, then saffron extract was added (2 g/500 ml), and they were stored at 8–10°C. The prepared products were put into presterilized glass bottles and sealed with crown caps.

2.5. Sensory Evaluation. The sensory evaluation of nutraceutical beverages was carried out using the method given by [24]. Three samples of nutraceutical beverage coded as SEBB 1, SEBB 2, and SEBB 3 were evaluated based on five sensory variables, namely, taste, aroma, color, appearance, and overall acceptability; samples were assessed and scored by a panel of 30 trained cuppers on a 10-point scale, with 1 indicating a very low preference and 10 indicating a very high preference as shown in Figure 2.

2.6. Animals and Experimental Design. Female nulliparous and nonpregnant albino rats aged 10 to 12 weeks and weighing 160–210 g were procured from Zakho University's primary animal house facility. The rats were housed in a filter-protected, air-conditioned room with a constant temperature of 21–25°C, 50–60% humidity, and a 12 h photoperiod. Three rats were kept in each cage and given unrestricted access to normal diet. All efforts were made to minimize both the number of animals used and unwanted stress or discomfort to the animals throughout the experiment. 15 rats were split into five groups at random. SEBB 1 was selected for further biological analysis due to the overall acceptability and taste scores: group 1 (control) rats served as controls; group 2 (CCl_4 only) rats were given 3 ml of CCl_4/kg/day in the feed; group 3 (saffron extract-based beverage (SEBB 1) only) rats were given 8 ml of SEBB 1/kg bw/day by oral injection seven times a week throughout the study; group 4 (SEBB 1+CCl_4) animals received 8 ml of SEBB 1/kg bw/day for 4 weeks before being given CCl_4 in the feed; and group 5 (CCl_4+SEBB 1) animals received CCl_4 with feed and then were treated with 8 ml of SEBB 1/kg bw/day starting from week 4 up to the end of the study. All rats were starved for 24 h after the final treatment on day

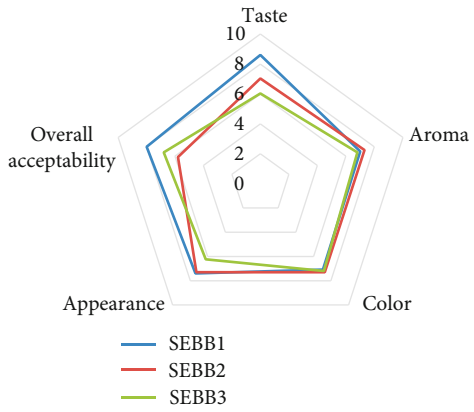

FIGURE 2: The results of the sensory evaluation for the nutraceutical beverage from saffron SEBB 1 containing 65 g of sucrose per 500 ml, SEBB 2 containing 17.5 g, and SEBB 3 containing 79.5 g.

62 but were allowed free access to water. Then, the animals were anesthetized by chloroform, a blood sample was collected intracardially and centrifuged at 4000 rpm for 15 min to get serum and kept at −80°C, and then, serum biochemical parameters were examined.

2.7. Study Parameters

2.7.1. Measurement of Study Parameters. The concentrations of blood glucose, total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, uric acid, blood urea, blood urea nitrogen, high-density lipoprotein, triglycerides, and cholesterol were evaluated using a Roche/Hitachi cobas system (model C-501/502). Sandwich ELISA kits were employed to evaluate total antioxidant capacity according to the manufacturer's instructions (Elabscience, USA).

2.7.2. Calculation of Study Parameters. The concentrations of globulin, very-low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C), and phospholipids were calculated as per the following equations [25–29]:

$$\begin{aligned}
\text{Globulin}\left(\frac{\text{g}}{\text{dl}}\right) &= \text{total protein concentration} \\
&\quad - \text{albumin concentration}, \\
\text{VLDL} - \text{C}\left(\frac{\text{mg}}{100}\text{ml}\right) &= \frac{\text{triglyceride concentration}}{5}, \\
\text{LDL} - \text{C}\left(\frac{\text{mg}}{100}\text{ml}\right) &= \text{total cholesterol} \\
&\quad - (\text{HDL} + 0.20(\text{TG})), \\
\text{Phospholipids}\left(\frac{\text{mg}}{100}\text{ml}\right) &= (\text{total cholesterol concentration} \times 0.89) \\
&\quad + 68.
\end{aligned} \tag{1}$$

2.8. Statistical Analysis. All analyses were carried out in triplicate, and the data were expressed as means ± standard deviations (SD). One-way analysis of variance (ANOVA) followed by Duncan's multiple range test was used to compare the treatments.

3. Results and Discussion

3.1. Quantification of Crocin and Safranal in Saffron Extract. To evaluate the quantity of the active compounds in saffron extract, HPLC analysis was carried out. The data showed that ethanol extracts of saffron contained 23.039 ppm crocin and 4.135 ppm safranal Figure 3. Mykhailenko et al. [30] reported a crocin concentration of 180–226 mg/g in saffron extract using the validated HPLC method, while the safranal concentration did not exceed 2.5 mg/g. In addition, the study of Vahedi et al. [31] also reported a considerable amount of crocin and safranal in saffron extract using HPLC analysis. Compounds such as safranal and crocin are known to be involved in redox activity, and they play an important role in reducing or increasing the activity of medicinal plants, fruits, and vegetables. Because of their redox activity, safranal and crocin are hydrogen donors, reducers, oxygen radical scavengers, and even metal-chelating agents [32]. Antioxidants are beneficial in the treatment and prevention of cardiovascular, cancer, and neurological diseases [11].

3.2. Sensory Evaluation. In order to select the preferred formulation in terms of consumer acceptability, samples of the three formulations of the saffron beverage were evaluated. Based on panelists' perceptions, SEBB 1 formulated with 65 g/500 ml of sucrose had the highest taste scores among the tested beverages, followed by SEBB 2 formulated with 17.5 g/500 g and SEBB 3 formulated with 79.5 g/500 ml of sucrose Figure 2.

According to the sensory analysis, all the formulations had an average aroma (average score of 7), reddish color (average score of 7.2), average appearance (average score of 7), and good overall acceptability; SEBB 2 and SEBB 3 were given low taste scores (average 6.5), and SEBB 1 had a very good taste (average score of 8). However, when the concentration of sucrose was increased up to 79.5 g/500 ml (SEBB 3), testers reported a strong taste in the mouth which affected their perceptions. Thus, SEBB 1 was the best formulation in terms of overall acceptability and taste scores and was selected for further biological analysis.

3.3. Effects of Nutraceutical Beverage on Total Antioxidant Capacity. Feeding rats food contaminated with CCl_4 led to a significant decrease in the concentration of indicators of total antioxidant capacity in the blood serum (Table 1). This decrease is attributed to several reasons, including an increase in the rate of consumption of superoxide dismutase, catalase, and glutathione, which is one of the most important endogenous antioxidants in removing free radicals resulting from oxidative stress, protecting cell membranes from free radical damage [33, 34].

Animals treated with the nutraceutical beverage made from saffron extract before or after receiving food tainted with CCl_4 had total antioxidant capacities of 1.40 mol/ml and 1.38 mol/ml, respectively, in groups G4 and G5. In the group of animals exposed to oxidative stress with CCl_4

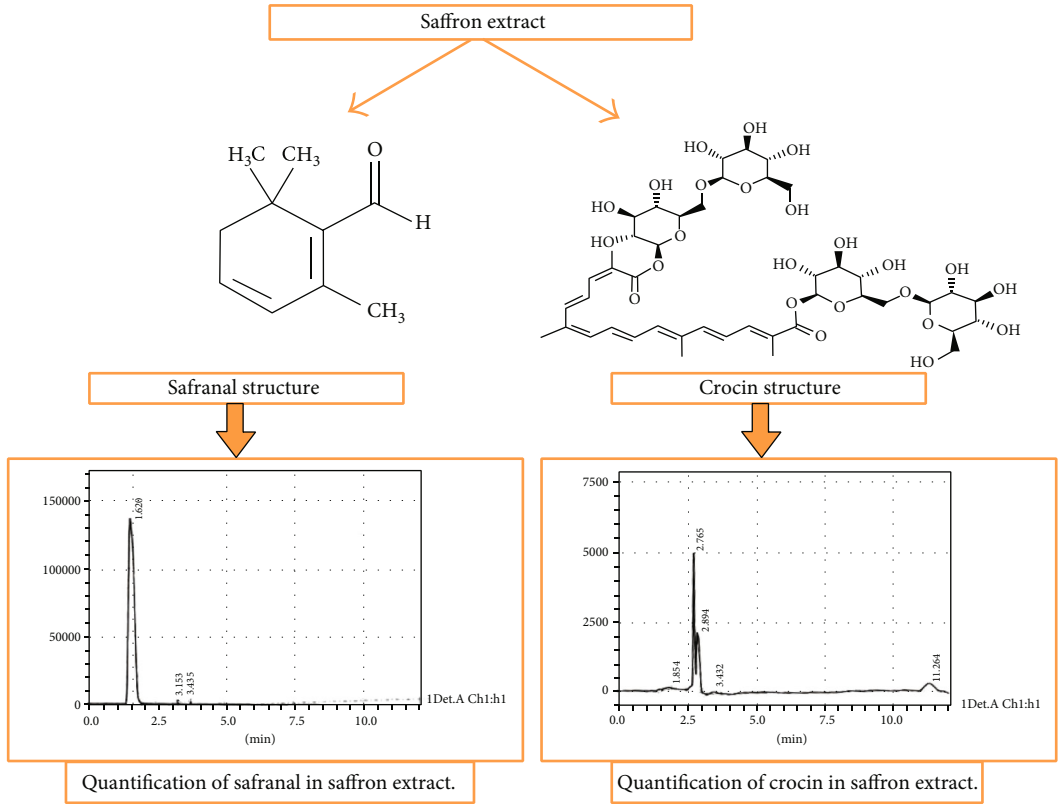

Figure 3: Concentration of crocin and safranal in saffron extract.

Table 1: Effects of nutraceutical beverage from saffron with 65 g/500 ml of sucrose (SEBB 1), on total antioxidant capacity, blood glucose, total protein, albumin, globulin, cholesterol, triglycerides, and phospholipid levels.

Groups	Total antioxidant capacity (μmol/ml)	Glucose (mg\dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Phospholipids (mg/100ml)
G1	1.52 ± 0.01^{a}	76.50 ± 1.35^{b}	6.83 ± 0.25^{a}	3.73 ± 0.15^{a}	3.10 ± 0.10^{a}	76.00 ± 3.60^{b}	67.00 ± 31.57^{b}	135.64 ± 3.20^{b}
G2	0.33 ± 0.03^{c}	169.53 ± 0.25^{a}	4.33 ± 0.05^{c}	2.10 ± 0.10^{c}	2.17 ± 0.15^{b}	235.00 ± 30.41^{a}	227.00 ± 6.08^{a}	277.15 ± 27.06^{a}
G3	1.58 ± 0.16^{a}	78.03 ± 1.59^{b}	6.36 ± 0.37^{ab}	3.73 ± 0.15^{a}	$2.\pm0.44^{ab}$	77.66 ± 2.08^{b}	74.00 ± 21.37^{b}	137.12 ± 1.85^{b}
G4	1.40 ± 0.61^{b}	79.00 ± 3.26^{b}	6.28 ± 0.02^{ab}	3.66 ± 0.15^{ab}	2.61 ± 0.17^{ab}	96.00 ± 13.45^{b}	84.66 ± 5.03^{b}	153.44 ± 11.97^{b}
G5	1.38 ± 0.66^{b}	75.23 ± 5.30^{b}	5.88 ± 0.55^{b}	3.40 ± 0.20^{b}	2.34 ± 0.60^{b}	128.00 ± 64.13^{b}	89.33 ± 9.29^{b}	181.92 ± 57.07^{b}

[a,b,c]Different letters within each column indicate significant differences ($p \leq 0.05$), and data are presented as the means ± standard deviation. G1: control; G2: carbon tetrachloride CCl_4 only; G3: SEBB 1only; G4: SEBB 1+ CCl_4; G5: CCl_4+SEBB 1; there were 3 female rats in each group of experiment.

(G2), the total antioxidant capacity was 0.33 mol/ml. When animals were treated with the nutraceutical beverage made from saffron extract, before or after giving them food contaminated with CCl_4, groups G4 and G5 had total antioxidant capacities of 1.40 mol/ml and 1.38 mol/ml, respectively; a significant increase was observed compared to that in the group of animals fed with food contaminated with CCl_4 (G2), which only had 0.33 mol/ml. This result is consistent with the findings of Hamdoon et al. [35], who observed an increase in glutathione level in male rats with hydrogen peroxide-induced oxidative stress after treatment with ginger extracts. The SEBB was able to raise the total antioxidant capacity because it contains the compounds crocin and safranal found in the saffron extract; these exogenous food antioxidants work to remove the constantly formed free radicals, protecting against the danger from these radicals as a line of defense against oxidative stress [36].

3.4. Effects of Nutraceutical Beverage on Blood Glucose. A significant increase in glucose concentration 169.53 mg\dl was found in the blood serum of G2 animals exposed to oxidative stress as a result of being fed with CCl_4-contaminated food, while animals treated with the saffron extract-based nutraceutical beverage before or after feeding them CCl_4-contaminated food had blood glucose levels of 79.000 mg/dl and 75.23 mg/dl, respectively, in groups G4

and G5. This is because H_2O_2 (formed as a result of oxidative stress) [37] leads to an increase in the generation of free radicals (ROS and RNS) that attack and destroy pancreatic beta cells, stimulating the process of lipid peroxidation, smashing RNA, and inhibiting RNA synthesis. Thus, inhibition of insulin and proinsulin secretion leads to an increase in blood glucose concentration, which leads to a halt in glucose breakdown and stimulates the processes of glucose formation and glycogenolysis [38, 39]. When rats were treated with SEBB before or after they were fed with food contaminated with CCl_4, a decrement in the blood serum glucose concentration of rats was observed [40, 41]. This could be due to the ability of these chemicals to slow down the rate of glucose production in cells [42], or because these substances may lead to a stimulation of the peripheral use of sugar from adipose and muscle tissues, directly or indirectly, by increasing sensitivity to insulin accompanied by a reduction in the glucose-building process [43].

3.5. Effects of Nutraceutical Beverage on Total Protein, Globulin, and Albumin. The total protein, globulin, and albumin levels were 4.33, 2.10, and 2.17 g/dl, respectively, in the group of animals exposed to oxidative stress with CCl_4 (G2). However, total protein, globulin, and albumin levels were 6.28, 3.66, and 2.61 and 5.88, 3.40, and 2.34 mg/dl, respectively, in G4 and G5, in the animals treated with the nutraceutical beverage made from saffron extract Table 1.

This may be attributed to the fact that animals exposed to oxidative stress resort to using alternative sources of energy in the body from fat and protein stores: catabolism of amino acids to produce energy increases and gluconeogenesis, the process of building glucose from noncarbohydrate sources [44]. On the other hand, the decrease may be due to the complications that occur in the kidneys due to diabetes, leading to what is known as diabetic nephropathy which is characterized by proteinuria, the loss of proteins through the urine [45]. Moreover, when animals were treated with SEBB before or after being fed with contaminated food, the total protein concentration increased significantly compared with that in animals that were not fed with the drink. The rise in these cases may be attributed to several reasons, including the effectiveness of antioxidants such as crocin and safranal found in the saffron extract. They remove free radicals, prevent protein oxidation, and stimulate the secretion of insulin from pancreatic beta cells. Alternatively, these compounds may reduce oxidative damage and its effect on the kidneys and glomeruli, reducing the filtration of proteins from the blood and their excretion with urine [46].

Albumin is the most sensitive type of blood protein to oxidative processes, which leads to a decrease in its blood serum concentration in patients exposed to oxidative stress for any reason. The decrease in albumin is due to the breakage of the peptide bonds between protein molecules due to the effect of oxidative stress and the generation of free radicals, which leads to the decomposition of albumin and a decrease in its concentration. This, in turn, leads to a defect in liver function, leading to a decrease in the synthesis of albumin and in its concentration in the blood serum, and immunoglobulins are part of serum proteins [47]. In the groups of animals treated with SEBB before or after giving them food contaminated with CCl_4, a significant improvement in the level of total protein, albumin, and globulin was observed compared to that in those fed with food contaminated with CCl_4 only without treatment.

3.6. Effects of Nutraceutical Beverage on ALT and AST. ALT and AST levels in the group of mice exposed to oxidative stress with CCl_4 were 47.00 and 80.00 U/L, respectively, in G2, while animals that were treated with the nutraceutical beverage originated from saffron extract before or after giving them food contaminated with CCl_4, the ALT and AST levels were 35.00 and 32.00 U/L and 37.66 and 44.33 U/L, respectively, in G4 and G5. The results showed a rise in the level of the enzymes ALT and AST in the animals fed with food contaminated with CCl_4 (Table 2). The occurrence of pathological infection of liver cells causes the release of these enzymes into the bloodstream, which leads to an increase in their concentration compared to the normal levels in the standard groups [48]. When SEBB was administered orally, ALT and AST levels were close to normal due to the active compounds present in the saffron extract, which contributed to reduce oxidative stress by inhibiting free radicals [48].

3.7. Effects of Nutraceutical Beverage on Creatinine, Uric Acid, Blood Urea, and Blood Urea Nitrogen. Animals fed with CCl_4-contaminated food had significantly higher levels of creatinine and urea in their blood serum than those in animals fed with a control diet. This increase in creatinine concentration could be due to the free radical damage, which causes a functional disorder of the cells in the inner layer of the glomerular capillary vessels, resulting in an increase in creatinine concentration in the blood and a decrease in creatinine excretion through urine [49]. The creatinine concentration was decreased in the groups of animals treated with the nutraceutical beverage; this is attributed to the role of active compounds that can reduce oxidative damage to renal cells and renal glomeruli. These antioxidant compounds, crocin and safranal, prevent renal enlargement and maintain the glomerular filtration rate within the normal range [50]. The high level of urea in the case of oxidative stress can also be explained by the loss of the direct source of energy and the animals resorting to the exploitation of proteins as an alternative source of energy, which results in the formation of large quantities of urea [51]. Treatment of animals with the nutraceutical beverage led to a significant decrease in urea concentration compared to that in the animals not given the drink. This may be attributed to the effectiveness of the antioxidants crocin and safranal in the drink, which operate to eliminate free radicals and prevent protein and amino acid oxidation, thus reducing the production of urea in the body [52]. Another reason may be that the active compounds can reduce oxidative damage to the renal cells and glomeruli and protect the liver from oxidative damage, thus reducing the disruption and degradation of proteins and lowering the concentration of urea in the blood

Table 2: Effects of nutraceutical beverage from saffron with 65 g/500 ml of sucrose (SEBB 1), on alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, uric acid, blood urea, blood urea nitrogen (BUN), high-density lipoproteins (HDL), very-low-density lipoprotein (VLDL-C), and low-density lipoprotein cholesterol (LDL-C) levels.

Groups	ALT (U/L)	AST (U/L)	Creatinine (mg/dl)	Uric acid (mg/dl)	Blood urea (mg/dl)	(BUN) (mg/dl)	HDL (mg/dl)	VLDL-C (mg/100ml)	LDL-C (mg/100ml)
G1	28.00 ± 1.00^{c}	16.00 ± 1.00^{d}	0.53 ± 0.02^{b}	2.50 ± 0.10^{b}	31.13 ± 1.10^{c}	14.10 ± 0.10^{b}	40.00 ± 5.00^{b}	13.40 ± 6.31^{b}	22.60 ± 8.91^{b}
G2	47.00 ± 3.00^{a}	80.00 ± 13.00^{a}	1.72 ± 0.10^{a}	6.86 ± 0.25^{a}	55.53 ± 3.50^{a}	29.00 ± 7.00^{a}	76.33 ± 5.13^{a}	$45.40\ 1.21^{a}$	$113.\pm36.03^{a}$
G3	27.00 ± 1.00^{c}	16.66 ± 2.08^{d}	0.53 ± 0.01^{b}	2.40 ± 0.10^{b}	32.53 ± 0.85^{c}	14.90 ± 0.43^{b}	42.66 ± 7.02^{b}	14.80 ± 4.27^{b}	13.33 ± 8.92^{b}
G4	35.00 ± 4.35^{b}	32.00 ± 5.29^{c}	0.55 ± 0.02^{b}	2.66 ± 0.15^{b}	36.70 ± 0.26^{b}	17.66 ± 2.08^{b}	$43.00\ 7.21^{b}$	16.93 ± 1.00^{b}	36.06 ± 15.28^{b}
G5	37.66 ± 1.52^{b}	44.33 ± 4.04^{b}	0.57 ± 0.02^{b}	2.60 ± 0.20^{b}	36.70 ± 0.10^{b}	20.33 ± 3.21^{b}	42.33 ± 17.21^{b}	17.86 ± 1.85^{b}	67.80 ± 73.78^{ab}

[a,b,c]Different letters within each column indicate significant differences ($p \leq 0.05$), and data are presented as the means ± standard deviation. G1: control, G2: carbon tetrachloride CCl_4 only; G3: SEBB 1only; G4: SEBB 1+CCl_4; G5: CCl_4+SEBB 1; there were 3 female rats in each group of experiment.

[52, 53]. A decrease in the concentration of uric acid was also noted due to its antioxidant role, as it has the ability to inhibit the process of lipid peroxidation and prevent the oxidation of LDL-C through the association of uric acid with ferrous ions (Fe^{2+}) or Cu^{2+}, stopping the Fenton reaction and then inhibiting the formation of free radicals [54].

3.8. Effects of Nutraceutical Beverage on Lipid Profile. The results showed a significant increase in the concentration of cholesterol, triglycerides, LDL-C, VLDL-C, and phospholipids (PL) in the blood serum of animals fed with a CCl_4-contaminated diet compared to the control group (Table 1). This could be due to an increase in the absorption of cholesterol by the intestine due to the increased activity of cholesterol acyltransferase. This enzyme is responsible for cholesterol absorption, which is stimulated by insulin deficiency as a result of the oxidative stress that affects the pancreatic beta cells under the influence of active classes of oxygen; CCl_4 increases the formation of ROS [55–57].

The increment in the level of LDL-C in the blood serum is due to the increase in the level of malondialdehyde caused by oxidative stress [58] or the oxidative stress generated by free oxygen radicals (ROS) leading to the release of the hormones epinephrine and norepinephrine. These two hormones activate the hormone-sensitive enzyme lipase, which is found in fat cells, causing the rapid hydrolysis of triglycerides and the release of fatty acids, thus increasing the level of LDL-C [59]. The high concentration of VLDL-C may be credited to the increment in the concentration of free radicals in the body as a result of oxidative stress, as these radicals destroy pancreatic beta cells and fatty tissue and then increase the release of free fatty acids that the liver uses in large quantities in the production of VLDL-C [60]. Alternatively, the reason for this may be the increase in oxidative stress as a result of the high concentrations of free radicals generated, which leads to a decrease in activity of the enzyme lipoprotein lipase found in the tissues of the body; this, in turn, leads to an imbalance in fat concentrations and a high concentration of triglycerides in the serum. The formation of VLDL-C then contributes to an increase in its concentration in the blood serum [61].

The high concentration of phospholipids may be due to an increase in phospholipid peroxidation, especially in the cell membranes, which leads to the transfer of phospholipids to the bloodstream; this occurs on exposure to oxidative stress as a result of the formation of free radicals and an increase in the concentration of malondialdehyde [62]. Feeding the animals with food contaminated with CCl_4 led to a significant decrease in HDL-C concentration in the blood serum compared with the control group. When animals were administered with the nutraceutical beverage orally before or after exposure to oxidative stress with CCl_4, there was a significant increase in HDL-C concentration compared to that in the animals that were not given the beverage. This is due to the active ingredients present in the drink, which improved the level of HDL-C to ratios close to normal. The high concentration of HDL-C may be attributed to the active compounds crocin and safranal working to remove free radicals and, significantly, types of active oxygen through their antioxidant activity and an increase in the activity of some enzymatic antioxidants such as catalase and superoxide dismutase in the liver [48]. However, when animals were fed the nutraceutical beverage orally before or after being fed with food contaminated with CCl_4, there was a decrease in cholesterol, triglycerides, VLDL-C, and phospholipids compared to groups fed with food contaminated with CCl_4 only.

This is due to the active groups (crocin and safranal) and their role in reducing the damage to the effective types of oxygen and in increasing the effectiveness of some enzymes that work against free radicals inside the body, such as the enzymatic antioxidant glutathione-S-transferase [63]. Another reason may be the effectiveness of these compounds to increase the activity of antioxidant enzymes which reduce oxidative stress (produced by food contaminated with CCl_4) and prevent the process of lipid peroxidation, which leads to a decrease in the concentration of VLDL-C in the blood serum [48–52].

4. Conclusion

Feeding rats with foods contaminated with CCl_4 resulted in an increase in glucose, ALT, AST, creatinine, urea, uric acid, total cholesterol, triglycerides, LDL-C, and VLDL-C and a decrease in total antioxidant capacity, total proteins, globulin, and albumin. Administration of a nutraceutical beverage made from saffron extract had favorable effects on oxidative stress and significantly boosted the antioxidant system. While anecdotal evidence has provided much of the basic knowledge about the health advantages of several nutraceuticals, scientific verification of efficacy, product standardization, stability, and safety is still needed, as is research into the metabolism and metabolites of nutraceutical beverages. In vitro and animal studies may be used to begin such investigations, but clinical studies may be required for essential proof. While most people can benefit from nutraceuticals, some may need to weigh the risks and benefits based on their genetics and lifestyle. Furthermore, nutraceutical antagonistic effects and nutraceutical-drug interactions demand special study.

Abbreviations

SEBB: Saffron extract-based beverage
CCl_4: Carbon tetrachloride
ppm: Parts per million
ROS: Reactive oxygen species
ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
VLDL-C: Very-low-density lipoprotein cholesterol
LDL-C: Low-density lipoprotein cholesterol
HDL-C: High-density lipoprotein cholesterol
PL: Phospholipids.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Additional Points

*Practical Applications.*Nutraceutical beverage of saffron production optimized contained 65 g of sucrose per 500 ml. The nutraceutical beverage of saffron ameliorated the increased activity of enzymes involved in liver and kidney function. The nutraceutical beverage of saffron improved the total antioxidant capacity, blood glucose, and lipid profile. Sensory evaluation illustrated that the nutraceutical beverage of saffron had the highest acceptability scores.

Ethical Approval

Ethical approval was obtained from the Ethics Committee of the College of Veterinary Medicine, Institutional Animal Care and Use Committee, University of Mosul, No. UM.VIT.2021.012.

Consent

Informed consent was obtained from all subjects before participating in this study.

Conflicts of Interest

The authors declared no conflict of interest.

Authors' Contributions

Qaswaa Yousif Jameel was responsible for the formal analysis, methodology, project administration, funding acquisition, validation, and writing of the original draft. Nameer Khairullah Mohammed was responsible for the data curation, formal analysis, methodology, project administration, supervision, resources, validation, and writing (review and editing). Mohammed Abdullah Ajeel was responsible for the data curation, methodology, and validation. All authors agreed with the publication of this paper.

References

[1] World Health Organization (WHO), "Carbon tetrachloride in drinking-water," WHO Guidelines for Drinking-water Quality, 2004.

[2] P. Balakumar, V. A. Chakkarwar, V. Kumar, A. Jain, J. Reddy, and M. Singh, "Experimental models for nephropathy," *Journal of the Renin-Angiotensin-Aldosterone System*, vol. 9, no. 4, pp. 189–195, 2008.

[3] E. A. Abdulrazak and Q. Y. Jameel, "Effect of spinach-derived glutathione against carbon tetrachloride-induced stress in rats," *Functional Foods in Health and Disease*, vol. 12, no. 8, pp. 442–454, 2022.

[4] A. Hermenean, A. Ardelean, M. Stan et al., "Protective effects of naringenin on carbon tetrachloride-induced acute nephrotoxicity in mouse kidney," *Chemico-Biological Interactions*, vol. 205, no. 2, pp. 138–147, 2013.

[5] B. M. Izzularab, M. Megeed, and M. Yehia, "Propolis nanoparticles modulate the inflammatory and apoptotic pathways in carbon tetrachloride-induced liver fibrosis and nephropathy in rats," *Environmental Toxicology*, vol. 36, no. 1, pp. 55–66, 2021.

[6] Z. Aghaei, S. M. Jafari, and D. Dehnad, "Effect of different drying methods on the physicochemical properties and bioactive components of saffron powder," *Plant Foods for Human Nutrition*, vol. 74, no. 2, pp. 171–178, 2019.

[7] S. Rahaiee, S. Moini, M. Hashemi, and S. A. Shojaosadati, "Evaluation of antioxidant activities of bioactive compounds and various extracts obtained from saffron (Crocus sativus L.): a review," *Journal of Food Science and Technology*, vol. 52, no. 4, pp. 1881–1888, 2015.

[8] G. Canet, C. Hernandez, C. Zussy, N. Chevallier, C. Desrumaux, and L. Givalois, "Is AD a Stress-Related Disorder? Focus on the HPA Axis and Its Promising Therapeutic Targets," *Frontiers in Aging Neuroscience*, vol. 11, pp. 1–9, 2019.

[9] Y. N. Sundukov, "First record of the ground beetle Trechoblemus postilenatus (Coleoptera, Carabidae) in Primorskii Krai," *Far Eastern Entomologiste*, vol. 165, 2016.

[10] A. S. Darwich, U. Aslam, D. M. Ashcroft, and A. Rostami-Hodjegan, "Meta-analysis of the turnover of intestinal epithelia in preclinical animal species and humans," *Drug Metabolism and Disposition*, vol. 42, no. 12, pp. 2016–2022, 2014.

[11] X. Su, C. Yuan, L. Wang et al., "Review article: the beneficial effects of saffron extract on potential oxidative stress in cardiovascular diseases," *Oxidative Medicine and Cellular Longevity*, vol. 2021, Article ID 6699821, 14 pages, 2021.

[12] C. Sun, S. H. Nile, Y. Zhang et al., "Novel insight into utilization of flavonoid glycosides and biological properties of saffron (Crocus sativusL.) flower byproducts," *Journal of Agricultural and Food Chemistry*, vol. 68, no. 39, pp. 10685–10696, 2020.

[13] K. Premkumar, C. Thirunavukkarasu, S. K. Abraham, S. T. Santhiya, and A. Ramesh, "Protective effect of saffron (Crocus sativus L.) aqueous extract against genetic damage induced by anti-tumor agents in mice," *Human and Experimental Toxicology*, vol. 25, no. 2, pp. 79–84, 2006.

[14] A. Bulman, N. M. D'cunha, W. Marx, A. J. McKune, R. Jani, and N. Naumovski, "Nutraceuticals as potential targets for the development of a functional beverage for improving sleep quality," *Beverages*, vol. 7, no. 2, p. 33, 2021.

[15] Q. Y. Jameel and N. K. Mohammed, "Protective rules of natural antioxidants against gamma-induced damage—a review," *Food Science & Nutrition*, vol. 9, no. 9, pp. 5263–5278, 2021.

[16] L. Shi, E. Kim, L. Yang et al., "Effect of a combined microwave-assisted drying and air drying on improving active nutraceutical compounds, flavor quality, and antioxidant properties of *Camellia sinensisL.* (cv. Longjing 43) flowers," *Food Quality and Safety*, vol. 5, pp. 1–7, 2021.

[17] Q. Y. Jameel and N. K. Mohammed, "Storage stability of yoghurts enriched with coriander (Coriandrum sativum l.) seeds extract," *Food Research*, vol. 5, no. 4, pp. 179–190, 2021.

[18] A. E. Özen, M. del Mar Bibiloni, A. Pons, and J. A. Tur, "Fluid intake from beverages across age groups: a systematic review," *Journal of Human Nutrition and Dietetics*, vol. 28, no. 5, pp. 417–442, 2015.

[19] I. Siró, E. Kápolna, B. Kápolna, and A. Lugasi, "Functional food. Product development, marketing and consumer acceptance–a review," *Appetite*, vol. 51, no. 3, pp. 456–467, 2008.

[20] A. Koul and S. K. Abraham, "Intake of saffron reduces γ-radiation-induced genotoxicity and oxidative stress in mice," *Toxicology Mechanisms and Methods*, vol. 27, no. 6, pp. 428–434, 2017.

[21] G. Mradu, S. Saumyakanti, M. Sohini, and M. Arup, "HPLC profiles of standard phenolic compounds present in medicinal

plants," *International Journal of Pharmacognosy and Phytochemical Research*, vol. 4, no. 3, pp. 162–167, 2012.

[22] M. S. B. Iahtisham-Ul-Haq, M. A. Randhawa, and M. Shahid, "Hepatoprotective effects of red beetroot-based beverages against CCl4-induced hepatic stress inSprague Dawleyrats," *Journal of Food Biochemistry*, vol. 43, no. 12, pp. 1–11, 2019.

[23] B. Renuka, S. G. Kulkarni, P. Vijayanand, and S. G. Prapulla, "Fructooligosaccharide fortification of selected fruit juice beverages: effect on the quality characteristics," *LWT- Food Science and Technology*, vol. 42, no. 5, pp. 1031–1033, 2009.

[24] M. Çam, N. C. Içyer, and F. Erdoğan, "Pomegranate peel phenolics: microencapsulation, storage stability and potential ingredient for functional food development," *LWT-Food Science and Technology*, vol. 55, no. 1, pp. 117–123, 2014.

[25] H. Goldenberg and P. A. Drewes, "Direct photometric determination of globulin in serum," *Clinical Chemistry*, vol. 17, no. 5, pp. 358–362, 1971.

[26] V. A. Buzanovskii, "Determination of proteins in blood. Part 1: determination of total protein and albumin," *Review Journal of Chemistry*, vol. 7, no. 1, pp. 79–124, 2017.

[27] P. W. Wilson, R. D. Abbott, R. J. Garrison, and W. P. Castelli, "Estimation of very-low-density lipoprotein cholesterol from data on triglyceride concentration in plasma," *Clinical Chemistry*, vol. 27, no. 12, pp. 2008–2010, 1981.

[28] T. Hirano, K. Nohtomi, S. Koba, A. Muroi, and Y. Ito, "A simple and precise method for measuring HDL-cholesterol subfractions by a single precipitation followed by homogenous HDL-cholesterol assay," *Journal of Lipid Research*, vol. 49, no. 5, pp. 1130–1136, 2008.

[29] J. Rosing, G. Tans, J. W. P. Govers-Riemslag, R. F. Zwaal, and H. C. Hemker, "The role of phospholipids and factor Va in the prothrombinase complex," *Journal of Biological Chemistry*, vol. 255, no. 1, pp. 274–283, 1980.

[30] O. Mykhailenko, I. Bezruk, L. Ivanauskas, and V. Georgiyants, "Comparative analysis of the major metabolites of Ukrainian saffron samples by HPLC," *Plant Foods for Human Nutrition*, vol. 76, no. 3, pp. 394–396, 2021.

[31] M. Vahedi, M. Kabiri, S. A. Salami, H. Rezadoost, M. Mirzaie, and M. R. Kanani, "Quantitative HPLC-based metabolomics of some Iranian saffron (*Crocus sativus* L.) accessions," *Industrial Crops and Products*, vol. 118, pp. 26–29, 2018.

[32] A. Kordzadeh, A. Ramazani Saadatabadi, and A. Hadi, "Investigation on penetration of saffron components through lipid bilayer bound to spike protein of SARS-CoV-2 using steered molecular dynamics simulation," *Heliyon*, vol. 6, no. 12, article e05681, 2020.

[33] H. Sies, "Findings in redox biology: from H_2O_2 to oxidative stress," *Journal of Biological Chemistry*, vol. 295, no. 39, pp. 13458–13473, 2020.

[34] P. Krishnamoorthy, S. Vaithinathan, A. Vimal Rani, and A. Bhuvaneswari, "Effect of Terminalia chebula fruit extract on lipid peroxidation and antioxidative system of testis of albino rats," *African Journal of Biotechnology*, vol. 6, no. 16, pp. 1888–1891, 2007.

[35] A. A. Hamdoon, M. S. Kalo, E. M. Al-Khashab, and S. M. Al-Katib, "The antioxidant effects of flavonoids and non flavonoid part extracted from ginger (Zingiber officinale) roots," *Rafidain Journal of Science*, vol. 20, no. 6, pp. 18–31, 2009.

[36] S. Mehri, K. Abnous, A. Khooei, S. H. Mousavi, V. M. Shariaty, and H. Hosseinzadeh, "Crocin reduced acrylamide-induced neurotoxicity in Wistar rat through inhibition of oxidative stress," *Iranian Journal of Basic Medical Sciences*, vol. 18, no. 9, pp. 902–908, 2015.

[37] N. E. Bolus, "Basic review of radiation biology and terminology," *Journal of Nuclear Medicine Technology*, vol. 45, no. 4, pp. 259–264, 2017.

[38] E. Park and A. Giacca, "Mechanisms underlying fat-induced hepatic insulin resistance," *Future Lipidology*, vol. 2, no. 5, pp. 503–512, 2007.

[39] S. U. Weber, J. K. Lodge, C. Saliou, and L. Packer, "Antioxidants," in *Handbook of Cosmetic Science and Technology, Third Edition*, vol. 5, no. 2pp. 301–310, CRC Press, 2009.

[40] M. C. de Oliveira, R. Sichieri, and A. S. Moura, "Weight loss associated with a daily intake of three apples or three pears among overweight women," *Nutrition*, vol. 19, no. 3, pp. 253–256, 2003.

[41] D. A. Mohamed, I. M. Hamed, and S. E. Mohammed, "Utilization of grape and apricot fruits by-products as cheap source for biologically active compounds for health promotion," *Egyptian Journal of Chemistry*, vol. 64, no. 4, pp. 2037–2045, 2021.

[42] M. E. Cam, A. N. Hazar-Yavuz, S. Yildiz et al., "The methanolic extract of *Thymus praecox* subsp. *skorpilii* var. *skorpilii* restores glucose homeostasis, ameliorates insulin resistance and improves pancreatic β-cell function on streptozotocin/nicotinamide-induced type 2 diabetic rats," *Journal of Ethnopharmacology*, vol. 231, pp. 29–38, 2019.

[43] A. M. Alkassar and B. A. A. Shaker Alaboudy, "Effect of added different levels of Graviola Leavs meal (Annona muricata L) on biochemical blood traits and bacteriological account in broilers chicken," *IOP Conference Series: Earth and Environmental Science*, vol. 735, no. 1, article 012023, 2021.

[44] D. N. Ross, "Examination of the Cardiovascular System," in *A Surgeons' Guide to Cardiac Diagnosis*, pp. 25–43, Springer, Berlin, Heidelberg, 1962.

[45] S.-T. Shittu, W. Oyeyemi, T. Lasisi, S.-S. Shittu, T. Lawal, and S. Olujobi, "Aqueous leaf extract of Ocimum gratissimum improves hematological parameters in alloxan-induced diabetic rats via its antioxidant properties," *International Journal of Applied & Basic Medical Research*, vol. 6, no. 2, pp. 96–100, 2016.

[46] D. Loru, A. Incani, M. Deiana et al., "Protective effect of hydroxytyrosol and tyrosol against oxidative stress in kidney cells," *Toxicology and Industrial Health*, vol. 25, no. 4-5, pp. 301–310, 2009.

[47] K. Pierzynowska, J. Woliński, B. Weström, and S. G. Pierzynowski, "Maternal immunoglobulins in infants—are they more than just a form of passive immunity?," *Frontiers in Immunology*, vol. 11, pp. 1–5, 2020.

[48] P. Fang, B. Dou, J. Liang, W. Hou, C. Ma, and Q. Zhang, "Quercetin reduces oxidative stress and apoptosis by inhibiting HMGB1 and its translocation, thereby alleviating liver injury in ACLF rats," *Evidence-Based Complementary and Alternative Medicine*, vol. 2021, Article ID 2898995, 14 pages, 2021.

[49] S. E. Lee, J. E. Jang, H. S. Kim et al., "Mesenchymal stem cells prevent the progression of diabetic nephropathy by improving mitochondrial function in tubular epithelial cells," *Experimental and Molecular Medicine*, vol. 51, no. 7, pp. 1–14, 2019.

[50] A. I. Chowdhury, M. A. Habib, and S. Ghosh, "Effect of saffron (Crocus sativus L) on common non communicable disease: review from current clinical findings," *Journal of Ayurvedic and Herbal Medicine*, vol. 7, no. 2, pp. 93–108, 2021.

[51] M. Estévez and Y. Xiong, "Intake of oxidized proteins and amino acids and causative oxidative stress and disease: recent scientific evidences and hypotheses," *Journal of Food Science*, vol. 84, no. 3, pp. 387–396, 2019.

[52] S. Nanda and K. Madan, "The role of safranal and saffron stigma extracts in oxidative stress, diseases and photoaging: a systematic review," *Heliyon*, vol. 7, no. 2, article e06117, 2021.

[53] Ö. N. Alayunt, L. Aksoy, Y. S. Karafakioğlu, and S. Sevimli, "Assessment of anti-inflammatory and antioxidant properties of safranal on CCI4-induced oxidative stress and inflammation in rats," *Anais da Academia Brasileira de Ciências*, vol. 91, no. 2, 2019.

[54] A. Negre-Salvayre, P. Guerby, S. Gayral, M. Laffargue, and R. Salvayre, "Version of record," vol. 33, 2019, https://www.sciencedirect.com/science/article/pii/S0891584919315746.

[55] S. Afzal, M. A. Sattar, E. J. Johns, and O. A. Eseyin, "Peroxisome proliferator-activated receptor agonist (pioglitazone) with exogenous adiponectin ameliorates arterial stiffness and oxidative stress in diabetic Wistar Kyoto rats," *European Journal of Pharmacology*, vol. 907, article 174218, 2021.

[56] H. S. Chae, H. J. Kim, H. J. Ko, C. H. Lee, Y. H. Choi, and Y. W. Chin, "Transcriptome analysis illuminates a hub role ofSREBP2in cholesterol metabolism by α-mangostin," *ACS Omega*, vol. 5, no. 48, pp. 31126–31136, 2020.

[57] J. S. Silveira, G. L. Antunes, D. B. Kaiber et al., "Reactive oxygen species are involved in eosinophil extracellular traps release and in airway inflammation in asthma," *Journal of Cellular Physiology*, vol. 234, no. 12, pp. 23633–23646, 2019.

[58] J. F. Lesgards, P. Durand, M. Lassarre et al., "Assessment of lifestyle effects on the overall antioxidant capacity of healthy subjects," *Environmental Health Perspectives*, vol. 110, no. 5, pp. 479–486, 2002.

[59] V. Hajhashemi, A. Ghannadi, and B. Sharif, "Anti-inflammatory and analgesic properties of the leaf extracts and essential oil of *Lavandula angustifolia* Mill," *Journal of Ethnopharmacology*, vol. 89, no. 1, pp. 67–71, 2003.

[60] P. Zhang, X. Zhang, J. Brown et al., "Global healthcare expenditure on diabetes for 2010 and 2030," *Diabetes Research and Clinical Practice*, vol. 87, no. 3, pp. 293–301, 2010.

[61] L. Ghiadoni, A. E. Donald, M. Cropley et al., "Mental stress induces transient endothelial dysfunction in humans," *Circulation*, vol. 102, no. 20, pp. 2473–2478, 2000.

[62] J. Elith, C. H. Graham, R. P. Anderson et al., "Novel methods improve prediction of species' distributions from occurrence data," *Ecography*, vol. 29, no. 2, pp. 129–151, 2006.

[63] G. H. Montgomery, D. H. Bovbjerg, J. B. Schnur et al., "A randomized clinical trial of a brief hypnosis intervention to control side effects in breast surgery patients," *JNCI Journal of the National Cancer Institute*, vol. 99, no. 17, pp. 1304–1312, 2007.