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Supplements of an aqueous combination of *Justicia adhatoda* and *Ocimum tenuiflorum* boost antioxidative effects and impede hyperlipidemia

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Abstract

Background: *Justicia adhatoda* and *Ocimum tenuiflorum*, locally known as bashok and tulsi, are two ethnomedicinally important herbs that have been using as dietary supplements for several therapeutic applications. This study evaluated the combined effect of both the herbs as an antioxidative and antihyperlipidemic agent.

Methods: Antihyperlipidemic effect was assessed in a high-fat diet-induced hyperlipidemic model in Wistar albino rats. The rats were treated orally with extracts of bashok (*J adhatoda*, 200 mg/kg bw), tulshi (*O tenuiflorum*, 200 mg/kg bw), and a combination of bashok and tulshi (50:50), as well as with a reference drug, atorvastatin (10 mg/kg/day), with or without high-fat diet for 14 days. The antioxidative effect was studied using established in vitro models. The studies were supported by experimentally testing the effects of the extracts on membrane stabilization and inhibition of protein denaturation.

Results: The results showed that the serum lipid profile was significantly decreased in the different treatment groups, with bashok having the greatest effect. Body weights, total serum protein, LDH, and relative liver and adipose tissue weights were markedly restored towards baseline values, the lowest atherogenic index being achieved with the combined extract. The combination treatment significantly enhanced total phenolic content and antioxidative capacity and greatly potentiated membrane stabilization, but inhibition of protein denaturation was not significantly affected.

Conclusion: The data demonstrate that a combination of *Justicia adhatoda* and *Ocimum tenuiflorum* could be developed as a food supplement with antioxidative and antihyperlipidemic benefits.

KEYWORDS

antihyperlipidemic, antioxidative capacity, high-fat fed diet-induced hyperlipidemia, *Justicia adhatoda*, membrane stabilization, *Ocimum tenuiflorum*

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1 | INTRODUCTION

Hyperlipidemia, the elevation of blood lipid and lipoprotein fraction, is characterized by elevated levels of serum total cholesterol (TC), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) and inadequate levels of high-density lipoprotein (HDL). These symptoms are highly predictive risk factors for coronary artery diseases (CADs), atherosclerosis, cerebral vascular diseases, and stroke, which are primary causes of death.[1,2] Arterial atherosclerosis (sclerero-hardening) is very common in the arterial network. It is a progressive and silent killer disease and is characterized by the formation of atherosclerotic plaques in the coronary arteries. This leads to reduced blood flow to the myocardium, resulting in CAD.[3] Atherosclerotic plaques are made up of lipids/fats, cholesterol, and other substances circulating in the blood.[4] The narrowing of arteries caused by the fibrous plaques limits the flow of oxygenated blood to parts of the body. Atherosclerosis is mostly asymptomatic and risk factors include abnormal cholesterol levels, familial hypercholesteremia, diabetes, cigarette smoking, high blood pressure, obesity, vitamin B6 deficiency, as well as an unhealthy diet.[4] What these risk factors have in common is that they all enhance inflammation and oxidative stress, vital components in the development and progression of atherosclerosis.[5] In severe cases, atherosclerosis results in myocardial infarction, peripheral artery disease, stroke or cardiovascular death.[4] Genetic disorders, sedentary behavior, and diets high in calories, saturated fat, and cholesterol largely contribute to the dyslipidemias seen in the developing world.[6] Primary hyperlipidemia may be treated with antilipidemic drugs, while the secondary form originating from renal lipid nephrosis, diabetes or hypothyroidism requires treatment of the causative disease rather than hyperlipidemia.[7]

The practice of using medicinal plants as food supplements or herbal drugs has become mainstream throughout the world because, compared to synthetic drugs, they are safer and have fewer side effects due to their natural origins. Medicinal plants already play an important role in the treatment of hypolipidemia by reducing lipid absorption in the intestine and inhibiting hepatic cholesterol biosynthesis.[8] Natural antioxidants such as vitamins, carotenoids and phenolics found in the higher plants have been shown to exhibit antioxidant activity and reduce lipid-associated chronic health problems.[9] It has also been reported that there is an inverse relationship between antioxidative status and ageing and the incidence of human diseases such as cancer, neurodegenerative disease, and atherosclerosis.[10]

Justicia adhatoda L. (bashok) is a tall dense evergreen shrub belonging to the family Acanthaceae. The species is distributed in the South Asia and Indo-China region. *Ocimum tenuiflorum* L. (tulsi) is an erect, bushy sub-shrub belonging to the family Lamiaceae. This species is widely distributed in the tropical and semitropical region of the Indian subcontinent and other Asian countries. Individual cases of the use of *J adhatoda* and *O tenuiflorum* as traditionally important medicinal herbs and preventives against numerous diseases have been reported.[11,12] In traditional medicine, *J adhatoda* has been used for a multitude of disorders including bronchitis, blood disorders, leprosy, leucoderma, thirst, asthma, vomiting, fever, loss of memory, heart troubles, jaundice, mouth troubles, tumors, sore-eye, and venereal disorders.[13] Many scientific studies have indicated that *J adhatoda* has antibacterial, antifungal, anti-asthmatic, antihistaminic, anti-inflammatory, anti-ulcer, antioxidative, antitubercular, antitussive, larvicidal, anti-Alzheimer, and hepatoprotective effects.[14] Correspondingly, *O tenuiflorum* has been used traditionally for the prevention and cure of the common cold, cough, headache, earache, fever, influenza, sore throat, bronchitis, asthma, colic pain, hepatic disorders, malarial fever, flatulence, fatigue, skin diseases, wounds, migraine headaches, insomnia, arthritis, diarrhea, digestive disorders, and night blindness, and as an antidote for snake bite and scorpion sting.[15] The plant has also been reported to possess a wide range of pharmacological properties including antioxidant, hepatoprotective, anti-stress, immunomodulating, anti-inflammatory, antiviral, antifungal, antipyretic, antibacterial, antidiuretic, antidiabetic, hypolipidemic and antimalarial effects, with a wide margin of safety.[15] Considering the enormous ethnomedicinal use and therapeutic value of bashok and tulsi, this research aimed to elucidate their individual and combined antioxidative and antihyperlipidemic properties for the purpose of developing food supplements.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

All chemicals and reagents used in this research were analytical grade unless specified. Folin-Ciocalteu Reagent (FCR), Na_2CO_3, gallic acid, ascorbic acid, potassium acetate, rutin, and sodium phosphate were purchased from Sigma-Aldrich, Germany. Other chemicals were purchased from Sigma-Aldrich.

2.2 | Collection and identification of plant samples

Justicia adhatoda L. leaves were collected from Banshkhali, Chittagong and *O. tenuiflorum* L. leaves were purchased from a commercial cultivator in Natore district, Bangladesh during the months of July and August 2015. Both the samples were identified and authenticated by Dr Sheikh Bokhtear Uddin, Taxonomist and Professor of the Department of Botany, University of Chittagong. The samples are preserved in the institutional herbarium with accession numbers *J adhatoda* L. SBU 10035 (CTGUH) and *O tenuiflorum* L. SBU 11021 (CTGUH) for future reference.

2.3 | Preparation of the plant extract

The leaves were thoroughly washed and shade-dried followed by oven drying at 40°C for one week. They were ground with a mechanical grinder to make a coarse powder, which was sieved

(mesh size #40) to separate large particles. The resulting powder (500 g) was suspended in distilled water (850 mL) and incubated in a water bath at 40°C for an hour. After an hour, the solution was filtrated through Whatman no.1 to collect the supernatant, which was then concentrated to one-tenth of the original volume using a rotatory evaporator (RE 200, Bibby Sterling). The final volume was subjected to lyophilization in a freeze dryer. The lyophilized powder was then transferred to the sample vial and preserved at 4°C until further use.

2.4 | Optimization of the extraction procedure

The crude extraction procedure was optimized using an established procedure.[16] Briefly, the dried samples were extracted with methanol 98% at 23 ± 0.5°C. To evaluate the effect of the progress variables, extraction time (T: 36-72 hours) and solvent-sample ratio (SSR: 2/1 to 4/1) expressed as milliliters of solvent per gram of dry sample, a central composition design was used. The extract was filtered using filter paper (Whatman, Grade 589/2) and the solvent was evaporated using a vacuum evaporator (RE 200, Bibby Sterling Ltd).

2.5 | Toxicity at acute levels

Wistar rats, procured from Biomedical Unit of Bangladesh Council of Scientific and Industrial Research (BCSIR), Chittagong, were maintained in standard laboratory conditions (23 ± 2°C and 55 ± 5% humidity) and the experimental procedure conformed with OECD guidelines. Five animals were used for each acute toxicity test and were fasted overnight prior to extract administration. Each group of animals was administrated a single oral dose of 0.5, 1.0, 1.5, and 2.0 g/kg BW of *J adhatoda*, *O tenuiflorum* and their combination. After drug administration, food was removed for further 3-4 hours. Each animal was closely observed during the first 30 minutes, then for the first 24 hours (with special attention given for the first 4 hours), and thereafter periodically for a period of 3 days to record any delayed toxicity. Careful observation of side-effects was made once daily, by recording changes in skin and fur, eyes, mucous membrane, circulatory and respiratory rate, and autonomic and CNS changes.[17] The effective therapeutic dose was calculated as one-tenth of the median lethal dose using the arithmetical method of Karber[5] in association with the Hodge and Sterner scale (LD_{50} > 2.0 g/kg).[18] The following equation was used for the determination of LD_{50}:

$$LD_{50} = LD_{100} - \sum (a \times b)/n$$

where n = total number of animals used in group, a = the difference between two successive doses of administered extract/substance, b = the average number of dead animals in two successive doses, and LD_{100} = lethal dose causing the 100% death of all test animals.

2.6 | Animal maintenance

Thirty white Wistar albino male rats aged 6-7 weeks (average weight 150-200 g) were procured from Biomedical Unit of Bangladesh Council of Scientific and Industrial Research (BCSIR), Chittagong. The animals were accommodated in polycarbonate cages, supplied with tap water and a standard pellet diet and exposed to a 12/12 hours light-dark cycle (50%-60% humidity) in a dedicated animal house. The study was carried out according to the International Guidelines for Care and Use of Laboratory Animals and approved by the Institutional Animal Ethics Committee of the Faculty of Biological Science of the University of Chittagong, Bangladesh (AERB/FBS/UC/02, 2015).

2.7 | High-fat diet-induced hyperlipidemia

A hyperlipidemic/atherogenic diet (high-fat diet) was given to all the experimental rats for 14 days to induce hyperlipidemia. The hyperlipidemic diet consisted of a pellet diet with egg yolk and coconut oil; it contained 20 g of fat/100 g of diet (19 g of butter oil and 1 g of soybean oil added to provide essential fatty acids) and provided 19.34 kJ/g of diet, with 7.74 kJ/g as fat. Control animals received a normal diet composed of carbohydrate 48.8 g, protein 21 g, fat 3 g, calcium 0.8 g, phosphorus 0.4 g, fiber 5 g, moisture 13 g, ash 8 g. The growth rate of the animals was monitored for the entire treatment period.

2.7.1 | Experimental design

The experimental animals were randomly divided into six groups consisting of five animals each.

Group I (normal control, NC) was treated with 0.3% w/v carboxymethyl cellulose (CMC).

Group II (hyperlipidemic control, HC) was treated with high-fat diet for 14 days.

Group III (*J adhatoda*/bashok control) was treated with 200 mg/kg BW bashok extract and high-fat diet for 14 days.

Group IV (*O tenuiflorum*/tulsi control) treated with 200 mg/kg BW tulsi extract and high-fat diet for 14 days.

Group V (combined extracts, 50:50 ratio) was treated with high-fat diet and extract 200 (100 + 100) mg/kg/day for 14 days.

Group VI (reference control) was treated with a reference drug, atorvastatin at 10 mg/kg/day, and a high-fat diet for 14 days. Atorvastatin and the extracts were suspended in 0.3% w/v CMC for ease of oral administration.

Throughout the study, body weights were recorded. At the end of a 14-day intervention period the animals were anesthetized with halothane and blood samples were collected by the heart puncture method. Collected blood was immediately centrifuged to separate the serum, which was then quickly processed for analysis of TC, triglycerides (TG), HDL, LDL and VLDL. Total serum protein and glycemic

index were estimated using established laboratory protocols. Relative organ weights for liver and adipose tissues were also measured.

2.8 | Antioxidative effects of the experimental extracts

2.8.1 | Estimation of total plant phenolics

The total phenolic content of the plant extract was measured by the Folin-Ciocalteu method.[19] The plant extract (0.5 mL) was added to 2.5 mL of 10 times diluted Folin-Ciocalteu reagent and 2.5 mL of 7.5% sodium carbonate solution. To complete the reaction, the mixture was incubated at 25°C for 20 minutes. The absorbance of the reaction mixture was read at 760 nm. To quantify total phenolics, the gallic acid standard curve was used and the results were expressed as milligrams of gallic acid equivalent (GAE)/g of dried extract.

2.8.2 | Estimation of total plant flavonoids

Total flavonoid content of the extract was estimated using the modified method of Kumaran and Karunakaran.[20] Briefly, 1 mL of extract solution at 200 µg/mL and rutin (standard) at different concentrations were added to test tubes, followed by 3 mL of methanol, then 200 µL of 10% aluminum chloride solution and finally 200 µL (1 M) of potassium acetate solution. Each reaction test tube was quickly diluted with 5.6 mL of distilled water, shaken and incubated in a water bath for 30 minutes at room temperature to complete the reaction. The optical density of the pink colored solution was spectrophotometrically read at 415 nm against a methanol blank. Total flavonoid content was expressed as rutin equivalents (RE) using the following equation:

$$C = (c \times V) / m,$$

where C = total flavonoid content of the extract in mg RE/g, c = concentration of rutin calculated from calibration curve (mg/mL), V = volume of the sample solution (mL), m = weight of the sample (g). All tests were done in triplicate.

2.8.3 | Total antioxidative potential

The total antioxidant potential of the experimental extracts were estimated by the method of Lin and Tang.[21] Briefly, 300 µL of extract (200 µg/mL) and ascorbic acid, 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were mixed in test tubes. The test tubes were incubated at 95°C for 90 minutes to complete reaction. The absorbance of the solution was measured at 695 nm using a spectrophotometer. The blank solution was prepared using 3 mL of reagent solution and 300 µL of the same solvent used for the sample preparation. Total antioxidant capacity was expressed as the number of equivalents of ascorbic acid using the equation:

$$A = (c \times V) / m,$$

where A = total antioxidant capacity, mg/g plant extract, in ascorbic acid equivalents (AA), c = concentration of ascorbic acid obtained from a calibration curve (mg/mL), V = volume of the sample solution (mL), and m = weight of the sample (g).

2.8.4 | Free radical scavenging assay using the DPPH method

Radical scavenging activity was determined using the DPPH method described by Sanja et al, with minor modifications.[17,22] Briefly, 2.5 mL of extract and reference drug at different concentrations were mixed with 1.5 mL of a solution of DPPH in methanol (0.4 mM) in a test tube. The mixture was incubated for 30 minutes in the dark at room temperature to complete the reaction. The optical density of the solution was measured at 517 nm, with methanol and ascorbic acid used as negative control and positive controls, respectively. Free radical scavenging activity (%) was calculated using the following formula:

$$[(\text{Absorbance of control} - \text{Absorbance of sample}) / \text{Absorbance of control}] \times 100.$$

2.8.5 | Inhibition of lipid peroxidation

The inhibition of lipid peroxidation was measured using a modified version of the method of Apak et al[23] With this method, the degree of lipid peroxidation was measured using thiobarbituric acid reacting substances. Briefly, rat brain was homogenized in ice-cold phosphate buffer (50 mM, pH 7.4) with 0.15 M KCl to produce a 1/10 homogenate. The homogenate was centrifuged at 4°C (10 000 ***g***, 15 minutes) and the supernatant was used as a liposome for in vitro lipid peroxidation assay. KCl (1 mL, 0.15 M), 0.5 mL of brain homogenates and different concentrations of plant extract were mixed and the peroxidation reaction was initiated with the addition of 100 mL of 0.2 mM ferric chloride. After incubation at 37°C for 30 minutes, the reaction was stopped by adding 2 mL of ice-cold HCl (0.25 N) containing 15% TCA, 0.38% TBA, and 0.5% BHT. The reaction mixture was heated at 80°C for 60 minutes. The samples were cooled and centrifuged, and the absorbance of the supernatants was measured at 532 nm. The percentage inhibition of lipid peroxidation was calculated by the formula:

$$\%\ \text{Inhibition} = [(\text{Absorbance of control- Absorbance of sample}) / \text{Absorbance of control}] \times 100.$$

2.8.6 | Inhibition of protein denaturation

The extract combination was studied using the albumin denaturation inhibition technique described by Mizushima et al and Sakat et al, with minor modifications.[24,25] Briefly, the reaction mixture consisted

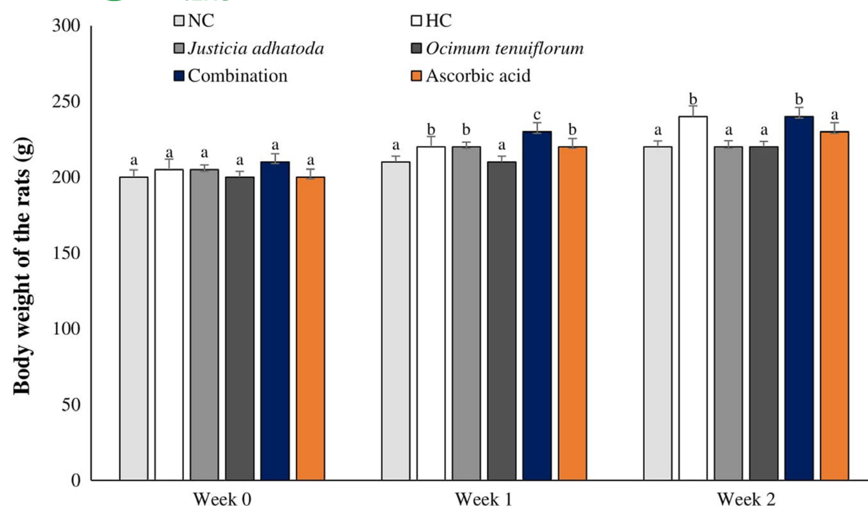

FIGURE 1 Effect of *Justicia adhatoda*, *Ocimum tenuiflorum* and a combined extract on the body weights of experimental animals. Data were expressed as mean ± SD. Data were analyzed by ANOVA (one-way analysis of variance) using the software SPSS (Statistical Package for Social Science, Version 22, IBM Corporation) followed by Tukey's post hoc test. $P < .05$ was considered significant. The values with superscript letters$^{a\text{-}c}$ on the bar graph indicate that they are significantly different from each other at least under the experimental conditions

of test extracts and a 1% aqueous solution of bovine albumin fraction, and the pH of the reaction mixture was adjusted using a small amount of 1 N HCl. The sample extracts were incubated at 37°C for 20 minutes and heated to 51°C for 20 minutes. After cooling the samples, the turbidity was measured at 660 nm (UV visible Spectrophotometer, Shimadzu Scientific Instruments Inc, UV-1280 multipurpose spectrophotometer). The experiment was performed in triplicate. The percentage inhibition of protein denaturation was calculated as follows:

$$\% \text{ Inhibition} = \left[\left(\text{Absorbance of control} - \text{Absorbance of sample} \right) / \text{Absorbance of control} \right] \times 100.$$

The plant extract concentration for 50% inhibition (IC_{50}) was determined from the dose-response curve.

2.8.7 | Determination of membrane stabilizing activity

This test was done according to the established method, with slight modifications.[26] Briefly, the test sample was made up of 0.03 mL of stock erythrocyte (RBC) suspension mixed with 5 mL of hypotonic solution (154 mM NaCl in 10 mM sodium phosphate buffer at pH 7.4) containing plant extract solutions at concentrations ranging from 25 to100 µg/mL. The control sample consisted of 0.03 mL of an RBC suspension mixed with hypotonic buffered solution alone. The reference drug indomethacin was tested at similar concentrations. The mixtures were incubated for 10 minutes at room temperature and centrifuged for 10 minutes at 1000 *g*, and the absorbance of the supernatant was measured spectrophotometrically at 540 nm. The percentage of haemolysis inhibition or membrane stabilization was calculated using following equation:

$$\% \text{Inhibition of haemolysis} = 100 \times \left[A_1 - A_2 / A_1 \right],$$

where A_1 = absorbance of hypotonic buffered solution alone and A_2 = absorbance of test/standard sample in hypotonic solution.

2.9 | Statistical analysis

All data are presented as the mean ± SD for five animals. Data were analyzed with Microsoft Xcel (Microsoft Office 2016) and one-way analysis of variance (ANOVA) using a statistical software package (SPSS for Windows, version 21, IBM Corporation) followed by Tukey's HSD multiple range post hoc test. Values were considered significantly different at $P < .05$. Microsoft Excel 2016 was also used for partial data analysis.

3 | RESULTS

3.1 | Effects on organ weights

Changes in body weights following treatment with *J adhatoda*, *O tenuiflorum*, the combined extracts, and atorvastatin are summarized in Figure 1. Compared to normal and reference controls, an insignificant effect of a high-fat diet on body weight was observed in the groups treated with *J adhatoda* and *O tenuiflorum*, but the increase of body weight was higher for the combination group. Organ weights (Table 1) and relative liver and adipose tissue weights of *J adhatoda*- and *O tenuiflorum*-treated groups were found to be lower than the normal control, hyperlipidemic control and reference control groups (Figure 2).

TABLE 1 Weights of liver and adipose tissue of different experimental groups

Group	Liver weight (g)	Adipose tissue weight (g)
NC	7.450 ± 0.023	1.930 ± 0.006
Hyperlipidemic control	8.390 ± 0.033	3.290 ± 0.064
Justicia adhatoda	6.960 ± 0.075	1.840 ± 0.017
Ocimum tenuiflorum	7.380 ± 0.110	2.090 ± 0.180
Combination	8.320 ± 0.076	1.370 ± 0.021
Reference control	7.630 ± 0.130	1.790 ± 0.075

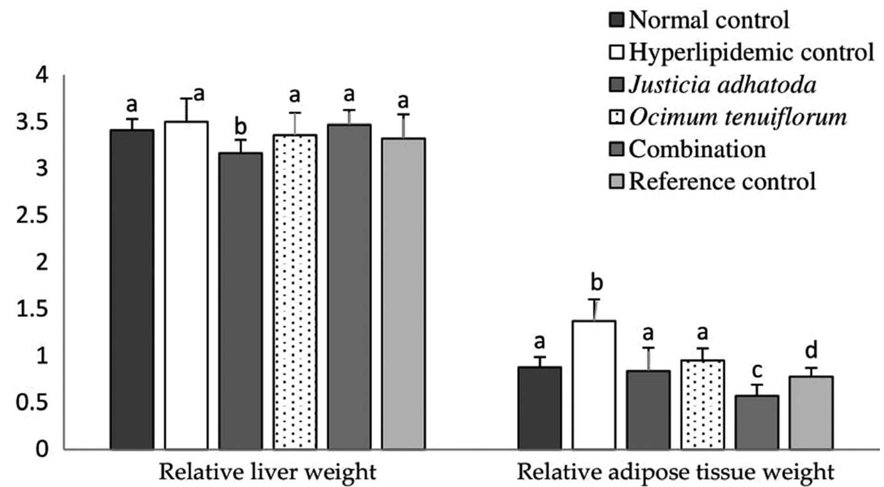

FIGURE 2 Effect of *Justicia adhatoda*, *Ocimum tenuiflorum* and a combined extract on relative organ weights of liver and adipose tissue of 25 animals. Data were expressed as mean ± SD. Data were analyzed by ANOVA (one-way analysis of variance) using the software SPSS (Statistical Package for Social Science, Version 22, IBM Corporation) followed by Tukey's Post Hoc Test. $P < .05$ was considered significant. The values with superscript letters [a-d]The level of significances between and among the treatment groups at least under the experimental conditions

TABLE 2 Serum lipid profiles of different intervention groups

Animal groups	TG (mg/dL)	TC (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	HDL (mg/dL)
NC	38.00 ± 2.52[a]	46.00 ± 1.53[a]	30.00 ± 1.00[a]	8.00 ± 0.76[a]	25.00 ± 1.53[a]
Hyperlipidemic control	58.00 ± 2.08[b]	57.00 ± 2.65[b]	38.00 ± 2.00[b]	10.00 ± 0.88[b]	15.00 ± 1.00[b]
Justicia adhatoda	29.00 ± 0.71[c]	45.00 ± 3.00[a]	25.00 ± 2.58[c]	5.00 ± 0.00[c]	26.00 ± 1.80[a]
Ocimum tenuiflorum	39.00 ± 1.53[a]	50.00 ± 3.51[c]	30.00 ± 3.03[a]	6.00 ± 1.00[d]	29.00 ± 2.00[c]
Combination	48.00 ± 2.00[d]	48.00 ± 5.56[c]	26.00 ± 1.59[c]	7.00 ± 0.00d	29.00 ± 3.00[c]
Reference control	38.00 ± 0.57[a]	48.00 ± 5.66[c]	20.00 ± 1.11[d]	7.00 ± 1.00[e]	34.00 ± 2.56[d]

Note: Effect of *Justicia adhatoda*, *Ocimum tenuiflorum* and combination on the serum lipid profiles. Data were expressed as mean ± SD. Data were analyzed by ANOVA (one-way analysis of variance) using the software SPSS (Statistical Package for Social Science, Version 22, IBM Corporation) followed by Tukey's post hoc test. $P < .05$ was considered significant. The values with superscript letters [a-e] in the table indicate significant differences between and among the groups.

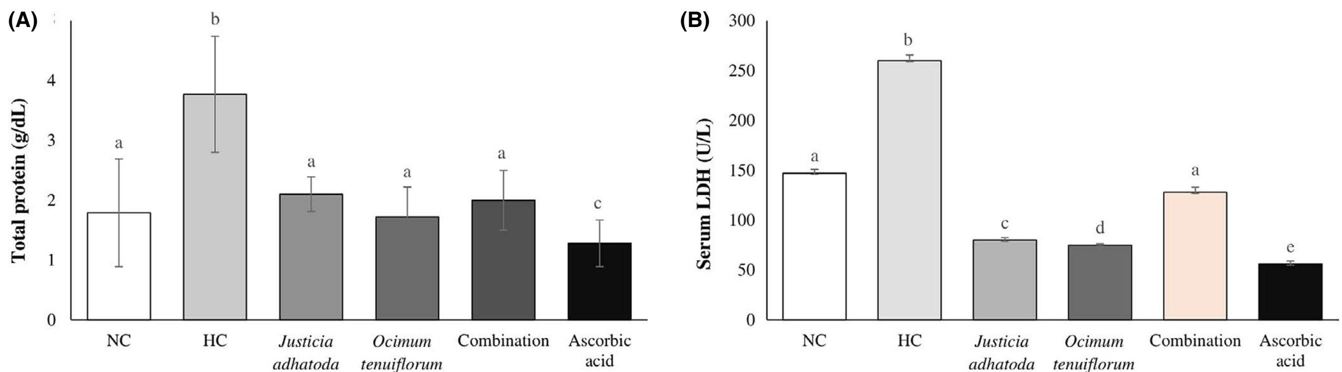

FIGURE 3 Effect of *Justicia adhatoda*, *Ocimum tenuiflorum* and a combined extract on total serum protein (A) and serum LDH (B). Data were expressed as mean ± SD for serum protein level. Data were analyzed by ANOVA (one-way analysis of variance) using the software SPSS (Statistical Package for Social Science, Version 22, IBM Corporation) followed by Tukey's post hoc test. $P < .05$ was considered significant. Superscript letters [a-c] above the bars denote the significant differences among the treatments

3.2 | Effects on serum lipid profile

The effects of the extracts on serum TC, TG, LDL, VLDL, and HDL in experimental rat models are shown in Table 2. Significant ($P < .05$) increases in TG, TC, LDL, and VLDL concentrations were noted in all hyperlipidemic control groups compared to the normal control groups and TG, TC, LDL, and VLDL were found to be significantly ($P < .05$) reduced in all the treatment groups compared to the hyperlipidemic control group. In contrast, the HDL concentration was found to be significantly ($P < .05$) increased in all treatment groups. However, lipid profiles were most effectively normalized in the *J adhatoda*-treated group compared with other groups. The highly increased levels of lactate dehydrogenase (LDH) in the hyperlipidemic control

group were significantly restored in all the treatment groups (Figure 3A). The effects of different extracts on LDH are shown in Figure 3B. The lowest atherogenic index (AI) was achieved with *J adhatoda*, while the *O tenuiflorum* and combination groups also had atherogenic indices lower than hyperlipidemic control (Figure 4).

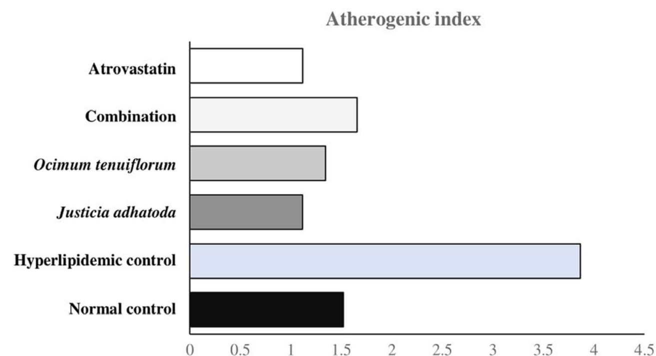

FIGURE 4 Effect of *Justicia adhatoda*, *Ocimum tenuiflorum* and a combined extract on atherogenic indexes for different types of treatments. Data for three consecutive weeks were analyzed using MS Office Excel 2016

3.3 | Total phenolic and total flavonoid content, total antioxidant capacity and DPPH radical scavenging assay

The antioxidative potential of the plant phenolic and flavonoid content and the total antioxidant capacity of the extracts and radical scavenging assessed by DPPH assay are summarized in Table 3. Flavonoid content (428.35 ± 15.30 g RE/100 g) and total antioxidant capacity were higher in *J adhatoda* than *O tenuiflorum*. The free radical scavenging effect in terms of inhibition concentration (IC_{50}) is shown in Figure 5 (the lower the IC_{50}, the higher the free radical scavenging potential). For all three treatments the IC_{50} values were statistically significant compared to the reference antioxidative agent, ascorbic acid (2.88 μg/mL). *Justicia adhatoda* had the lowest

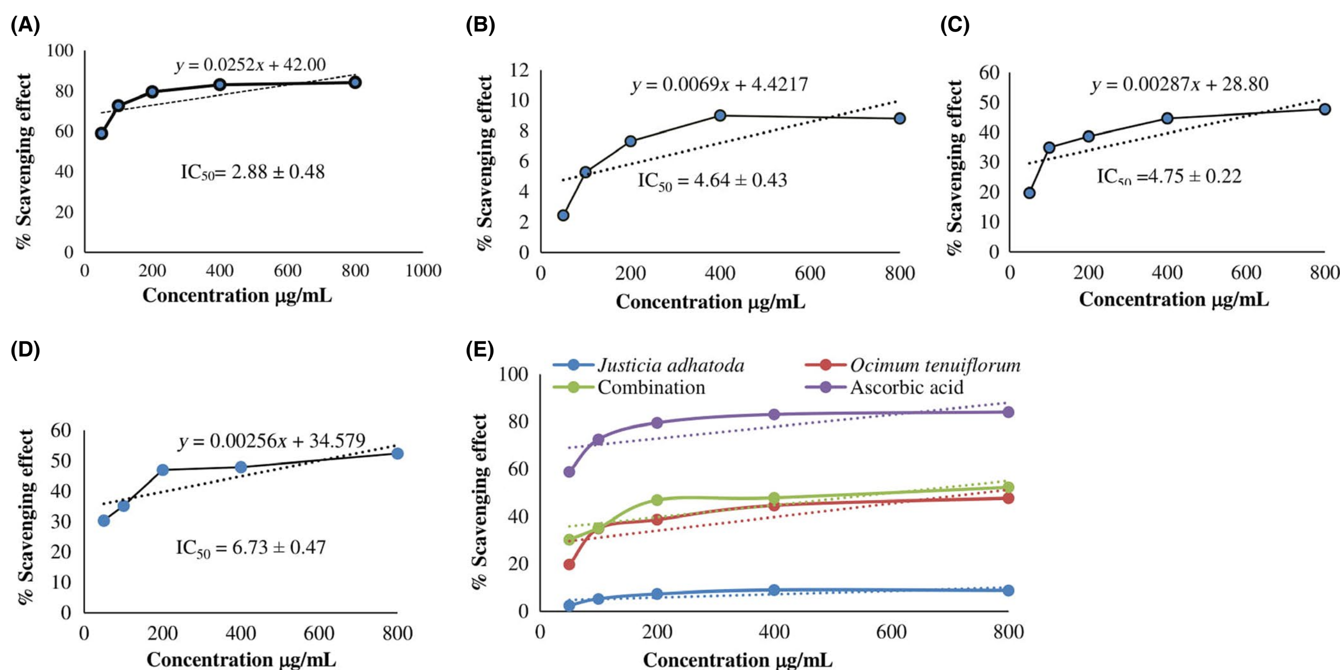

FIGURE 5 Antioxidative capacity of *Justicia adhatoda*, *Ocimum tenuiflorum* and a combined extract. The graphs show DPPH free radical scavenging activity of the different treatment groups. (A) Reference antioxidative agent ascorbic acid; (B) *Justicia adhatoda*; (C) *Ocimum tenuiflorum*; (D) A combination of *Justicia adhatoda* and *Ocimum tenuiflorum*; (E) A comparison of the scavenging effects among the treatments. Data are presented as mean ± SD

TABLE 3 Total phytochemical content of plant extracts

Phytochemical and antioxidative parameters	Justicia adhatoda	Ocimum tenuiflorum
Total phenolic	9.430 ± 0.063 g GAE/100 g	12.300 ± 0.289 g GAE/100 g
Total flavonoid	428.350 ± 15.300 g RE/100 g	256.650 ± 17.550 g RE/100 g
Total antioxidant	90.330 ± 4.040 g AA/100 g dry extract	32.330 ± 8.080 g AA/100 g of dry extract

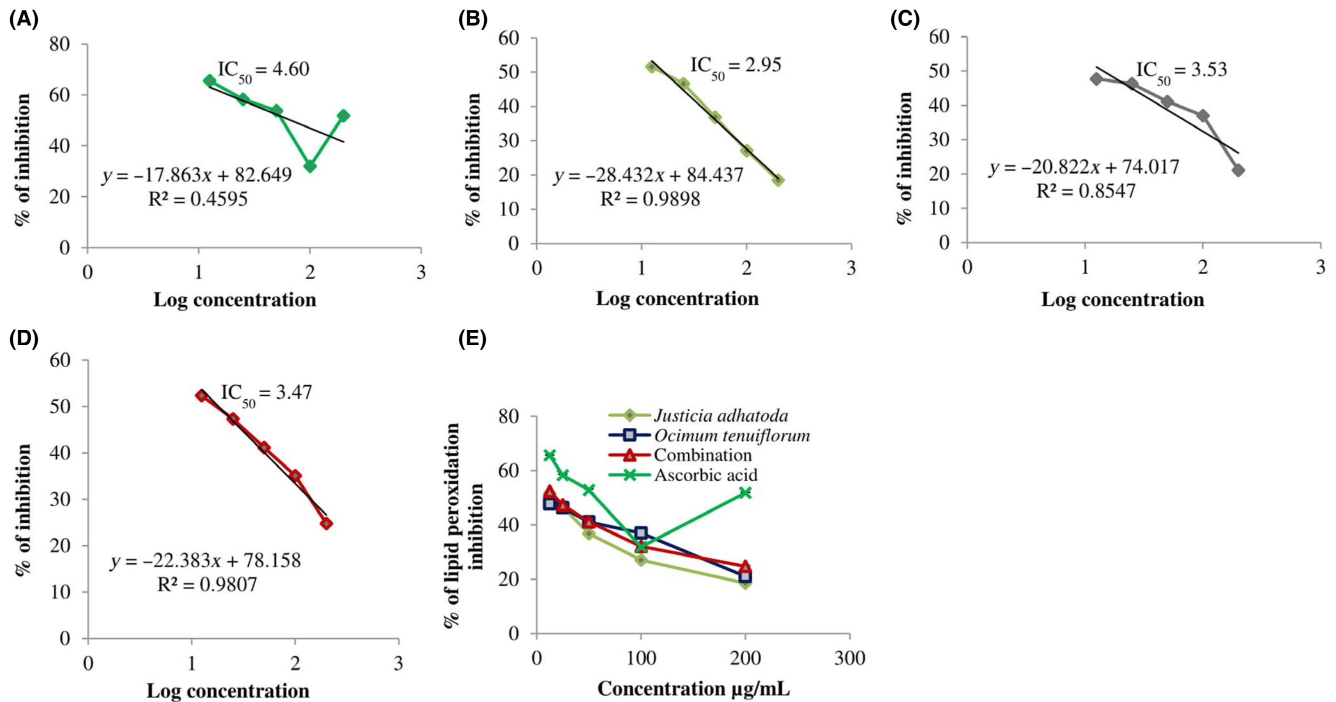

FIGURE 6 Lipid peroxidation capacity of *Justicia adhatoda*, *Ocimum tenuiflorum* and a combined extract. (A) Reference agent catechin; (B) *Justicia adhatoda*; (C) *Ocimum tenuiflorum*; (D) A combination of *Justicia adhatoda* and *Ocimum tenuiflorum*; (E) A comparison of the inhibition among the treatments. Data are presented as mean ± SD

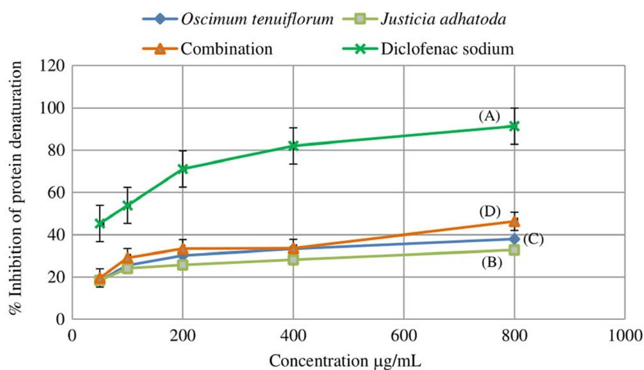

FIGURE 7 Effects of *Justicia adhatoda*; *Ocimum tenuiflorum* and their combination on protein denaturation. (A) Reference agent diclofenac sodium; (B) *Justicia adhatoda*; (C) *Ocimum tenuiflorum*; (D) A combination of *Justicia adhatoda* and *Ocimum tenuiflorum*. Data are presented as mean ± SD. Data were analyzed using Microsoft Office Excel 2016

IC_{50} value (4.64 µg/mL), and therefore the highest free radical scavenging capacity, while *O tenuiflorum* and the combination had IC_{50} values of 4.75 and 6.73 µg/mL, respectively.

3.4 | Inhibition of lipid peroxidation

The effect of the plant extracts on non-enzymatic lipid peroxidation in rat brain homogenate is shown in Figure 6. The extracts inhibited lipid peroxidation induced by hydrogen peroxide and the increase in inhibition was correlated with increasing concentrations of the extract. *Justicia adhatoda* was the most effective extract with an IC_{50} value of 2.95 µg/mL. However, all the IC_{50} values were significantly lower than that of reference standard (+)-catechin (4.60 µg/mL).

3.5 | Inhibition of protein denaturation

A dose dependent effect was found for all the treatments. At the highest concentration of the extracts, 800 µg/mL, a percentage inhibition of protein denaturation of 38.03 ± 2.71% was seen with *O tenuiflorum*, 32.82 ± 6.93% with *J adhatoda* and 46.29 ± 5.34% with the combination of both extracts. The values were statistically significantly different ($P < .05$) from that of the reference drug diclofenac sodium, which showed 91.40 ± 3.58% inhibition of protein denaturation. The results are summarized in Figure 7.

3.6 | Membrane stabilizing effects

The erythrocyte membrane stabilization potential of the extracts was studied. The extract concentration ranged from 0.75 to 3 mg/mL (w/v) and dilutions of these solutions (0.25, 0.50, 0.75, and 1 mL/mL) were applied to test the extracts' potential to protect the rat erythrocyte membrane against lysis induced by hypotonic solution. In the experimental assay, *O tenuiflorum*, *J adhatoda* and combination extracts showed 59.10 ± 2.06, 32.40 ± 1.26, 33.40 ± 3.02%

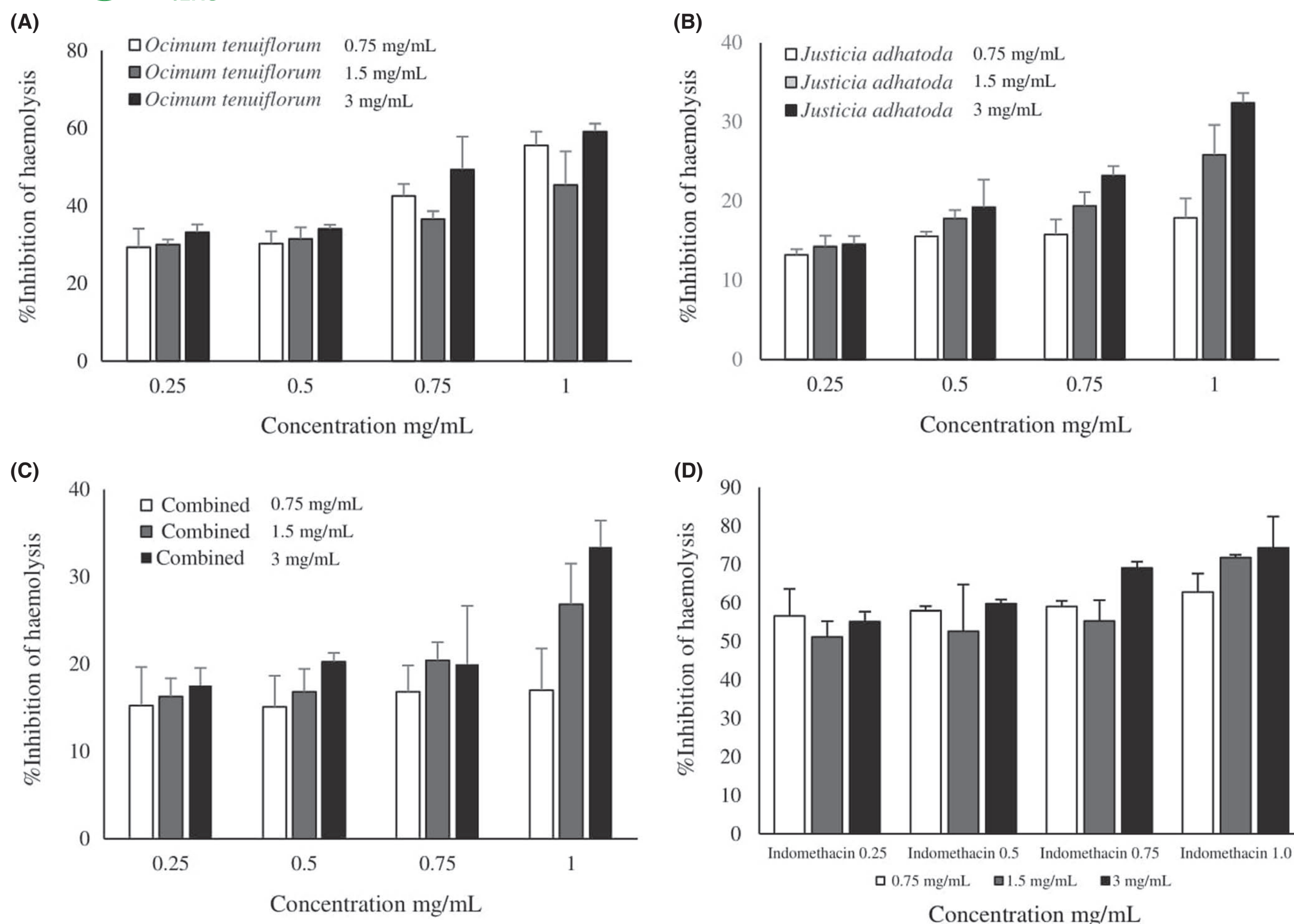

FIGURE 8 Membrane stabilizing (percentage inhibition of hemolysis) effect of *Justicia adhatoda*, *Ocimum tenuiflorum* and combination. (A) *Ocimum tenuiflorum*; (B) *Justicia adhatoda*; (C) A combination of *Justicia adhatoda* and *Ocimum tenuiflorum*; (D) Reference agent indomethacin. Data are presented as mean ± SD. Data were analyzed using Microsoft Office Excel 2016

inhibition of hemolysis, respectively, at the highest concentrations, and the values were statistically significant ($P < .05$) compared with that (74.35 ± 8.09%) of the reference drug indomethacin. The results are summarized in Figure 8.

4 | DISCUSSION

Hyperlipidemia is a well-known risk factor for cardiovascular diseases, especially atherosclerotic CAD, which is one of the major causes of premature death globally.[27] Recent studies have revealed that the increased risk of coronary heart disease is associated with an increase in blood serum TC, LDL cholesterol, and TG and a decrease in HDL cholesterol concentration.[28,29] Cholesterol feeding has often been used to elevate serum and tissue cholesterol levels to assess hypercholesterolemia-related metabolic disturbances in different animal models.[30] The abnormally high concentration of serum lipids is mainly due to the increase in free fatty acids mobilization from the peripheral depots.[31] Preclinical studies demonstrate that hyperlipidemia promotes the accumulation of LDLs in the arterial wall, thereby advancing endothelial dysfunction, which eventually develops into atherosclerosis and congestive heart diseases.[32]

The potential effects of several herbal combinations in healing various ailments including diabetes, atherosclerosis, allergic rhinitis, and rheumatoid arthritis have been proved experimentally.[33] For instance, a combination (of roots of ashwagandha, rhizomes of ginger, and young mulberry leaves) treatment provided to NIDDM human subjects showed a remarkable attenuation in blood glucose, TG, TC, and LDL/VLDL cholesterol.[34] In addition, combinations of the roots of *Glycyrrhiza glabra*, *Withania somnifera*, *Asparagus racemosus*, and *Chlorophytum borivilianum* and the seeds of *Sesamum indicum* have been found to possess significant hypocholesterolemic and antioxidant potential.[33] Likewise, a recent investigation led by Zhang et al[34] revealed that the combination of the root of *Salvia miltiorrhiza* Bge. and the fruit of *Crataegus pinnatifida* Bge. var. major NEBr have an antiatherosclerotic ability to lower lipid concentrations and to protect endothelial function, as well as possessing anti-inflammatory properties.

In this study the antihyperlipidemic effects of two plant extracts and their combination were tested in diet-induced hyperlipidemic Wistar albino rats. The rats were fed on high-fat diet to

create hyperlipidemia. The body weights of the hyperlipidemic control groups were significantly higher than the normal control group, probably due to the accumulation of fat, which is decreased after plant extract ingestion. The attenuation of TC by the combination aqueous extract was analogous to its reduction of the LDL cholesterol fraction in serum, which is the target of several antihyperlipidemic drugs. Some study reports suggest that the cholesterol-lowering effect of the aqueous extract of herbal formulations could be the result of rapid catabolism of LDL cholesterol.[35] HDL cholesterol is reported to have a preventive function against atherogenesis. In our study, the herbal combination also increased the HDL cholesterol, thus exhibiting antihyperlipidemic action. The decrease in serum TC and triglyceride levels in the different treatment groups may be due to decreased cholesterogenesis and fatty acid synthesis. Serum LDH is usually increased due to cardiac damage.[36] LDH and LDL decreased in the extract-treated groups, particularly the *J adhatoda* group, signifying its potential antihyperlipidemic effect; the effect of the combination treatment was moderate. Subramani et al[37] reported a significant increase in serum LDH levels in high-fat diet fed rats and higher activity of this enzyme has been found in response to oxidative stress induced by a high fat diet. The enzyme was restored to normal levels on administration of their plant extract, *Premna integrifolia*. They concluded that the remarkable decrease in LDH enzyme activity elicited by the plant extract demonstrated a cardio-protective effect comparable with that of atorvastatin, which significant reduces LDH activity. Measurement of LDH activity in blood is considered to be a diagnostic marker for certain cardiovascular diseases and it is a sensitive marker of myocardial infarction.[37]

The AI has been used to assess cardiovascular and atherosclerotic risk. People with high AI have a higher risk of coronary heart disease than those with low AI. Triglyceride (TG) and high-density lipoprotein-cholesterol (HDL-C) in AI reflect the balance between the atherogenic and antiatherogenic lipoproteins. Our research consistently identified the highest AI in the high-fat diet hyperlipidemic group, and this was greatly reduced in all three treatment groups, clearly indicating an effect.[36]

Total serum protein is usually increased in hyperlipidemic conditions. In many studies, a high fat diet is reported to result in oxidative stress, which is partially responsible for increasing total serum protein. In this study, total serum protein was measured as a non-specific marker because the initial period of cholesterinosis in blood serum is marked by an increase in total proteins, mainly albumin and globulin.[38] Gross body weight is, as expected, significantly increased though the intake of a high-cholesterol diet in albino rats. When the extracts were co-administered with the high-cholesterol diet, the levels of lipids and total serum proteins were significantly reduced and the AI was decreased, thereby confirming the antihyperlipidemic efficacy of the extracts.

Recent correlative relationship studies of total phenolic contents, particularly total flavonoids with antihyperlipidemic and antioxidant effects, of different plants provide evidence of the usefulness of plant phenolics in managing hyperlipidemia. Subsequent studies also suggest a physiological effect of flavonoids in the prevention of atherosclerosis and other coronary heart disease.[35] Flavonoids elicit responses that confirm this, making liver cells more efficient at removing LDL-C from blood by increasing the LDL-C receptor densities in liver and binding to apolipoprotein B. In this study, the *J adhatoda* extract had the highest flavonoid content and consistently showed antihyperlipidemic effects. Besides, the generation of free radicals in the form of reactive oxygen or nitrogenous species is currently a 'hot topic' in hyperlipidemia studies. The DPPH free radical scavenging assay is one of the most reliable methods of evaluating the antioxidative potential of natural resources; with this method an IC_{50} value of less than 1000 μg/mL (cut off value) is accepted as a potentiality important index.[39] In this study, the IC_{50} values for *J adhatoda* and the other treatments closely matched that of the standard antioxidative agent ascorbic acid, which further supports their role in enhancing the scavenging of free radicals. Additionally, reactive oxygen species produced by several different pathways cause metabolic processes that have pathophysiological consequences such as lipid peroxidation leading to damage to DNA, peroxidation of protein and degeneration of cells.[40-42] Researchers have postulated that plant products with significant antioxidative capacity can directly or indirectly lessen the extent of, or stop, the lipid peroxidation.[43] In this study, the plant extracts were evaluated for their capacity to inhibit non-enzymatic lipid peroxidation using rat brain homogenate and the results are reported as the inhibition concentrations (IC_{50}). The ability of the studied extracts, especially *J adhatoda*, to inhibit lipid peroxidation confirms their antioxidative action and consequently their potential to elicit antihyperlipidemic effects. These findings agree with results from recent studies showing that *J adhatoda* possesses antioxidative effects.[44,45] Additional support for our present findings comes from two other studies that have reported the antioxidative action of *O tenuiflorum*.[46,47]

Dyslipidemia accompanies increases in plasma triglyceride and atherogenic LDL cholesterol and deceases in HDL cholesterol. Investigations of cellular lipid profiles are essential to evaluate the changes in membrane lipid composition of hypertensive subjects due to their abnormal cellular lipid synthesis and metabolism,[48] and pharmacologic and nutritional interventions that normalize membrane abnormalities and restore blood pressure will be a very useful addition to these studies. Dyslipidemia research therefore requires a thorough evaluation of membrane lipid composition and microviscosity measurements of particular membrane domains.[49] The capacity of aqueous plant extracts to help reduce high fat-fed dyslipidemia by optimizing membrane stabilization can then be evaluated in experimental models.

5 | CONCLUSIONS

Antioxidative effects are considered to be pivotal in biological systems. Aqueous extracts of two plants were evaluated in in vitro and in vivo models for their antioxidative role in hyperlipidemia.

The study also examined associated biological effects such as anti-inflammatory activity, protein denaturation inhibition and inhibition of lipid peroxidation. The combination of the two plant extracts was found to be effective but did not prove to be better than the individual extracts in all regards. The antioxidative effects of the plants suggest their usefulness in advancing research aimed at elucidating and establishing the mechanisms behind the results obtained so far. Molecular studies using the same samples could evaluate the potential of the combined extracts as effective food supplements despite the variations in efficiency seen in the present animal model. Therefore, further studies are suggested to identify appropriate supplementary quantities and dosages of the combined extracts.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

IIC, AH, MAR and MHB carried out the experiments. IIC, MAR, AH, DH, WAE, AAM, MAH, JT and TBB participated in the analysis and interpretation of the data, and drafted the manuscript. MAR, JT and MAH coordinated the study, revised the manuscript and approved the final version to be submitted for publication. All authors read and approved the final manuscript.

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REFERENCES

1. Nelson RH. Hyperlipidemia as a risk factor for cardiovascular disease. *Prim Care*. 2013;40(1):195-211.
2. Patel D, Patel K, Patel U, et al. Assessment of lipid lowering effect of Sida rhomboidea. Roxb methanolic extract in experimentally induced hyperlipidemia. *J Young Pharm*. 2009;1:233-238.
3. Mohale D, Dewani A, Saoji A, Khadse C. Antihyperlipidemic activity of isolated constituents from the fruits of *Lagenaria siceraria* in albino rats. *Int J Green Pharm*. 2008;2(2):104-107.
4. Wu X, Wang TT, Prior RL, Pehrsson PR. Prevention of atherosclerosis by berries: the case of blueberries. *J Agric Food Chem*. 2018;66(35):9172-9188.
5. Hackam DG, Anand SS. Emerging risk factors for atherosclerotic vascular disease: a critical review of the evidence. *JAMA*. 2003;290(7):932-940.
6. Arsenault BJ, Perrot N, Couture P. Does lifestyle contribute to disease severity in patients with inherited lipid disorders?*Curr Opin Lipidol*. 2017;28(2):177-185.
7. Suzuki T, Suzuki Y. Virus infection and lipid rafts. *Biol Pharm Bull*. 2006;29(8):1538-1541.
8. Koriem KMM. Antihyperlipidemic activity of the medicinal plants among Kadazan and Dusun communities in Sabah, Malaysia: a review. *Asian Pac J Trop Biomed*. 2014;4(10):768-779.
9. Xu DP, Li YA, Meng X, et al. Natural antioxidants in foods and medicinal plants: extraction, assessment and resources. *Int J Mol Sci*. 2017;18(1):96.
10. Duarte MCT, Figueira GM, Sartoratto A, Rehder VLG, Delarmelina C. Anti-Candida activity of Brazilian medicinal plants. *J Ethnopharmacol*. 2005;97(2):305-311.
11. Ravi P, Elumalai A, Eswaraiah MC, Kasarla R. A review on Krishna tulsi, *Ocimum tenuiflorum* Linn. *Int J Res Ayurved Pharm*. 2012;3(2):291-293.
12. Dhankhar S, Kaur R, Ruhil S, Balhara M, Dhankhar S, Chhillar A. A review on *Justicia adhatoda*: a potential source of natural medicine. *Afr J Plant Sci*. 2013;5(11):620-627.
13. Hossain MT, Hoq MO. Therapeutic use of *Adhatoda vasica*. *Asian J Med Biol Res*. 2016;2(2):156-163.
14. Kapgate SM, Patil AB. *Adhatoda vasica*: a critical review. *Int J Green Pharm*. 2018;11(04):S654-S662.
15. Joshi RK, Setzer WN, Da Silva JK. Phytoconstituents, traditional medicinal uses and bioactivities of Tulsi (*Ocimum sanctum* Linn.): a review. *Am J Essent Oil Nat Prod*. 2017;5(1):18-21.
16. Rincón C, Montoya J, Gómez G. Optimizing the extraction of phenolic compounds from *Bixa orellana* L. and effect of physicochemical conditions on its antioxidant activity. *J Med Plant Res*. 2014;8(46):1333-1339.
17. Al-Araby SQ, Rahman MA, Chowdhury MAH, et al. *Padina tenuis* (marine alga) attenuates oxidative stress and streptozotocin-induced type 2 diabetic indices in Wistar albino rats. *S Afr J Bot*. 2020;128:87-100.
18. Zaoui A, Cherrah Y, Mahassini N, Alaoui K, Amarouch H, Hassar M. Acute and chronic toxicity of Nigella sativa fixed oil. *Phytomedicine*. 2002;9(1):69-74.
19. Verza SG, Kreinecker MT, Reis V, Henriques AT, Gonzales OG. Avaliação das variáveis analíticas do método de Folin-Ciocalteu para determinação do teor de taninos totais utilizando como modelo o extrato aquoso de folhas de *Psidium guajava* L. *Quím Nova*. 2007;30(4):815-820.
20. Kumaran A, Karunakaran R. In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. *Food Sci Technol*. 2007;40(2):344-352.
21. Lin JY, Tang CY. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food Chem*. 2007;101(1):140-147.
22. Sanja SD, Sheth NR, Patel NK, Patel D, Patel B. Characterization and evaluation of antioxidant activity of *Portulaca oleracea*. *Int J Pharm Pharm Sci*. 2009;1(1):74-84.
23. Apak R, Güçlü K, Demirata B, et al. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. *Molecules*. 2007;12(7):1496-1547.
24. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *J Pharm Pharmacol*. 1968;20(3):169-173.
25. Sakat SS, Juvekar AR, Gambhire MN. In vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. *Int J Pharm Pharm Sci*. 2010;2(1):146-155.
26. Shinde U, Kulkarni K, Phadke A, et al. Mast cell stabilizing and lipoxygenase inhibitory activity of *Cedrus deodara* (Roxb.) Loud. wood oil. *Indian J Exp Biol*. 1999;37(3):258-261.
27. Han X, Shen T, Lou H. Dietary polyphenols and their biological significance. *Int J Mol Sci*. 2007;8(9):950-988.
28. Liu X, Yu S, Mao Z, et al. Dyslipidemia prevalence, awareness, treatment, control, and risk factors in Chinese rural population: the Henan rural cohort study. *Lipid Health Dis*. 2018;17(1):119.
29. Lee JS, Chang PY, Zhang Y, Kizer JR, Best LG, Howard BV. Triglyceride and HDL-C dyslipidemia and risks of coronary heart

disease and ischemic stroke by glycemic dysregulation status: the strong heart study. *Diabetes Care*. 2017;40(4):529-537.
30. Brown TM, Bittner V. Biomarkers of atherosclerosis: clinical applications. *Curr Cardiol Rep*. 2008;10(6):497-504.
31. Ebbert JO, Jensen MD. Fat depots, free fatty acids, and dyslipidemia. *Nutrients*. 2013;5(2):498-508.
32. Gofman JW, Lindgren F, Elliott H, et al. The role of lipids and lipoproteins in atherosclerosis. *Science*. 1950;111(2877):166-171.
33. Andallu B, Radhika B, Suryakantham V. Effect of aswagandha, ginger and mulberry on hyperglycemia and hyperlipidemia. *Plant Foods Human Nutr*. 2003;58(3):1-7.
34. Zhang J, Liang R, Wang L, Yang B. Effects and mechanisms of Danshen-Shanzha herb-pair for atherosclerosis treatment using network pharmacology and experimental pharmacology. *J Ethnopharmacol*. 2019;229:104-114.
35. El-Beshbishy HA, Singab ANB, Sinkkonen J, Pihlaja K. Hypolipidemic and antioxidant effects of *Morus alba* L. (Egyptian mulberry) root bark fractions supplementation in cholesterol-fed rats. *Life Sci*. 2006;78(23):2724-2733.
36. Høstmark AT, Lunde MS, Haug A. Increased serum triglycerides and reduced HDL cholesterol in male rats after intake of ammonium chloride for 3 weeks. *Lipid Health Dis*. 2013;12(1):92.
37. Subramani C, Rajakkannu A, Rathinam A, et al. Anti-atherosclerotic activity of root bark of *Premna integrifolia* Linn. in high fat diet induced atherosclerosis model rats. *J Pharm Anal*. 2017;7(2):123-128.
38. Kulabukhov V, Voloshin P, Kostiukovskaia L, Bozhko G. Changes in the serum protein composition in experimental hypercholesterolemia. *Ukr Biokhim Zh (1978)*. 1987;59(2):27-33.
39. Chew AL, Jessica JJ, Sasidharan S. Antioxidant and antibacterial activity of different parts of *Leucas aspera*. *Asian Pac J Trop Biomed*. 2012;2(3):176-180.
40. Hu EC, He JG, Liu ZH, et al. High levels of serum lactate dehydrogenase correlate with the severity and mortality of idiopathic pulmonary arterial hypertension. *Exp Ther Med*. 2015;9(6):2109-2113.
41. Baptista FI, Henriques AG, Silva AMS, et al. Flavonoids as therapeutic compounds targeting key proteins involved in Alzheimer's disease. *ACS Chem Neurosci*. 2014;5(2):83-92.
42. Nimmi I, Jahan IA, Hossain MH, Uddin MB, Rana MS, Haq MM. A comparative study on antioxidant properties of two phyllanthus species growing in Bangladesh. *Dhaka Univ J Pharm Sci*. 2012;11(2):191-197.
43. Grotto D, Maria LS, Valentini J, et al. Importance of the lipid peroxidation biomarkers and methodological aspects for malondialdehyde quantification. *Quim Nova*. 2009;32(1):169-174.
44. Khan AM, Bhadauria S, Yadav R. Phytochemical Screening and Antioxidant activity of extract of different parts of Adhatoda vasica. *Res J Pharm Technol*. 2019;12(12):5699-5705.
45. Saran N, Anandharaj B, Bupesh G, Vasanth S, Surendhar PA. In vitro antioxidant potential of *Justicia adhatoda* leaf extracts against 1, 1-diphenyl picryl hydrazyl, hydroxyl, and nitrous oxide free radicals. *Drug Invention Today*. 2019;12(8):1736-1740.
46. Sharma J, Khurana N, Sharma N, Garg R. Phytochemical evaluation and antioxidant screening studies of *Ocimum tenuiflorum* Linn seeds. *Asian J Pharm Clin Res*. 2017;10(16):76.
47. Sankhalkar S, Vernekar V. Quantitative and qualitative analysis of phenolic and flavonoid content in *Moringa oleifera* Lam and *Ocimum tenuiflorum* L. *Pharm Res*. 2016;8(1):16-21.
48. Dominiczak AF, Davidson AO, Bohr DF. Plasma membrane in hypertension: microviscosity and calcium stabilization. *Hyper Res*. 1994;17(2):79-86.
49. Zicha J, Kuneš J, Devynck MA. Abnormalities of membrane function and lipid metabolism in hypertension: a review. *Am J Hypertens*. 1999;12(3):315-331.

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