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Effects of green synthesized calcium oxide nanoparticles from extracts of *Citrullus colocynthis* on body weight, plasma atherogenic index, and histology of liver and stomach of high-fat-diet-fed rats

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Abstract: *Citrullus colocynthis* has been used to treat obesity and hyperlipidemia. Nanoparticles, due to their nano size, phytochemical adsorption, improved systemic absorption and high bioavailability, can potentially improve the bioactivity of *C. colocynthis*. The present research focused on studying the effects of green synthesized calcium oxide nanoparticles (CaONPs) from *C. colocynthis* fruit extracts (CCFE) on the body weight, the atherogenic index of plasma (AIP), and the histopathology of male albino rats. Ethyl-α-d-glucopyranoside was the major constituent of CCFE, along with phenols and fatty acids. The total phenolic and flavonoid contents of CCFE were 203.52 and 173.56 mg/g, respectively. Male albino rats (n=36) were divided into six groups (six rats per each group). Rats in different groups received a normal diet, a high-fat diet (HFD), HFD with lovastatin (10 mg/kg), HFD with CCFE (20 mg/kg), HFD with CaONPs (2 mg/kg), and HFD with a synergistic solution of CCFE (10 mg/kg) and CaONPs (1 mg/kg), respectively. When CaONPs were administered in combination with CCFE, significant weight-lowering activity (36.36%), improved cardiovascular health as per AIP ($0.12\pm0.01_b$), and fewer disturbances in liver function parameters were observed. Histological analysis at the end of the experiment showed that CaONPs were not toxic to the gastric mucosa and were slightly toxic to hepatocytes. It can be concluded that CaONPs synthesized from CCFE can be a potent weight-lowering and antilipidemic agent with fewer side effects.

Keywords: calcium oxide nanoparticles; *Citrullus colocynthis*; weight-lowering; atherogenic index of plasma; histopathology

Abbreviations: Alkaline phosphatase (ALP); alanine transaminase (ALT); aspartate transaminase (AST); atherogenic index of plasma (AIP); calcium oxide nanoparticles (CaONPs); C. colocynthis fruit extracts (CCFE); high-fat diet (HFD); high-density lipoprotein-cholesterol (HDL-c); triglycerides (TG)

INTRODUCTION

Obesity is the accumulation of excessive fat in the body. It is expected that obesity will affect 20% of the world's population by the end of 2030. It is mainly caused by excessive calorie intake and low physical activity [1]. Obesity is a metabolic disorder that is interlinked with hyperlipidemia, diabetes, and cardiovascular disorders [2]. Fat accumulates around the belly and visceral organs of obese people. The fat accumulation around the liver enhances insulin resistance and increases the risk of cardiovascular diseases and related complications due to the disturbed physiology of the liver, kidneys, and heart [3]. There is an increased risk of heart failure in obese individuals due to disturbed hemodynamics, hormonal imbalance, and myocardial dysfunction [4]. A decrease in weight can reverse the negative effects of obesity. Weight loss can also help manage complications related to diabetes, chronic kidney disease, and hypertension [5].

Hyperlipidemia is a metabolic disorder that is increasing at a tremendous rate due to poor food choices and a sedentary lifestyle. According to a recent combined report of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS), high levels of blood lipids are the leading cause of hypertension and cardiovascular diseases [6]. High lipid concentrations in the blood can block the arteries through the formation of plaques and the deposition of cholesterol. These plaques disturb the flow of blood, causing an increase in blood pressure that has negative

effects on the eyes, kidneys, and heart [7]. To avoid cardiovascular disorders, blood lipid concentrations are maintained by using antilipidemic drugs, especially statins. The statins can reduce lipid concentrations but may cause myopathy and other side effects [8]. Several medicines for the treatment of hyperlipidemia that are available on the market are expensive and have side effects; the most manifest side effects being disturbed hepatic biochemical parameters [9]. Another problem associated with the available statin antilipidemic drugs is their pre-systemic degradation and clearance from the digestive tract without being absorbed [10]. Thus, a study has reported 88% of pre-systemic clearance of the antilipidemic drug atorvastatin and its very low (12%) bioavailability [11]. There is therefore an urgent need for new therapeutic agents for the treatment of hyperlipidemia with better systemic availability and fewer side effects [9,10,12].

Nanoparticles are used as carriers for improving drug delivery and the bioavailability of therapeutic agents. Nanoparticles can adsorb therapeutics on their surface and can pass through membranous barriers, allowing for improved bioavailability of the bioactive molecules adsorbed on their surface [13]. In one study, the deposition of atorvastatin on nanocrystals led to improved bioavailability as compared to atorvastatin alone [11]. Sericin nanoparticles fabricated with genipin, a chemical compound found in the fruit extract of *Genipa americana*, cross-linked with atorvastatin, showed significant antilipidemic activity and negligible toxicity [14]. The ginger-derived phytochemical 6-gingerol was able to stabilize and enhance the antilipidemic potential of polymeric nanoparticles due to the suppression of pre-systemic removal and better bioavailability over a 24-h period [15]. The nanoparticles loaded with polysaccharides from *Ulva fasciata* were found to be more antilipidemic as compared to fluvastatin. Histopathological analysis of the rat liver also proved that polysaccharide-fabricated nanoparticles were less toxic to hepatocytes [16]. Plant extracts contain several bioactive secondary metabolites that have an antilipidemic potential and can also stabilize metallic nanoparticles. Green synthesized nanoparticles from plant extracts with a known antilipidemic potential have proved to be more competitive antilipidemic agents and less toxic than herbal extracts [17].

Citrullus colocynthis is an important medicinal plant used to treat obesity and hyperlipidemia in traditional medicinal cultures. The fruit extracts of *C. colocynthis* can decrease lipid concentrations without disturbing the biochemical parameters of the liver [18]. Extracts of *C. colocynthis* fruit can decrease fat deposition around vital organs, including the kidneys, liver, and pancreas. It can also improve blood lipid concentrations by decreasing triglycerides, total cholesterol, and low-density lipoprotein cholesterol (LDL-c), as well as by increasing high-density lipoprotein cholesterol (HDL-c) in hyperlipidemic rats [19]. Experiments with commercial layer hens revealed that *C. colocynthis* seed extracts can enhance laying capacity by decreasing lipid concentrations and reduce mortality rates by enhancing immune responses [20,21].

Keeping in view the antilipidemic potential of *C. colocynthis* and the considerable pharmacokinetic capability of nanoparticles, the present study was designed to investigate the possible antilipidemic effects of green synthesized calcium oxide nanoparticles (CaONPs), *C. colocynthis* fruit extracts (CCFE), and synergistic solutions (SynS) of CaONPs on high-fat-diet-fed hyperlipidemic rats. We hypothesized that green synthesized nanoparticles could enhance the antilipidemic activity of *C. colocynthis* by improving the delivery of phytochemicals fabricated on their surface from *C. colocynthis* fruit extracts. No study has documented the antilipidemic potential of green synthesized CaONPs.

MATERIALS AND METHODS

Ethics statement

Albino male Wistar rats (*Rattus norvegicus*) were kept in accordance with departmental ethical committee (DEC) guidelines established by Mirpur University of Science and Technology (MUST), Azad Jammu and Kashmir (AJ&K), Pakistan. Approval (730/DEC/BOT/2021 of July 25, 2021) from the DEC was received in advance of the experiments. Global standard operating protocols and regulations for laboratory rats were applied. Prior to testing, rats were housed for 10 days as follows: humidity was manually kept at 60% and the temperature was set at 25°C. The dark and light periods were set to 12 h, respectively. Rats were provided with food and water *ad libitum* during the experiments.

Crude extraction and characterization of *C. colocynthis* fruit extracts (CCFE)

C. colocynthis plants were collected from Bhimber (32° 28' 0" N and 75° 6' 0" E), Pakistan. The identified herbarium no. MUST/BOT/MUH-1283 was submitted to the herbarium of the Botany Department, Mirpur University of Science and Technology, Pakistan. Fruits were separated, kept in the shade for 2 months to dry, and ground to a fine powder. Crude extraction was performed by the maceration method. To analyze the volatile constituents of CCFE, gas chromatography-mass spectrometry (GC-MS) was carried out as described [22], using a DB5 MS column (30 m × 0.25 mm × 0.25 mm) and GC 7890A (Agilent, California, USA) and MS 5975C (Agilent, California, USA) equipment. To calculate the total flavonoids, the Folin-Ciocalteu method was used [23]. For total phenolic contents, the flavonoid-aluminum complex protocol was followed [24].

Green synthesis and characterization of calcium oxide nanoparticles (CaONPs)

The CaONPs were synthesized from CCFE by calcination at 700°C [25]. Characterization was performed by UV-Vis spectroscopy in the range of 250 to 500 nm using a spectrophotometer (UV-1900i, Biotechnology Medical Service, China). The size and shape were studied by transmission electron microscopy (TEM) with the help of a microscope (JEM-ARMTM 200 JEOL, Japan). The structural properties were elucidated by X-ray diffraction (XRD) following the drop-casting method and using diffractometer (JEOL-JSX-3201M, Japan) with a radiation source of 40 kV and in the 2θ range of 20°C to 70°C. Fourier-transform infrared spectroscopy (FTIR) examined the fabrication of different phytochemicals using IRAffinity-1S MIRacle 10 (Shimadzu, Japan); the wavenumber range was 400-4000 cm^{-1}.

Research design

The experimental studies lasted 8 weeks. Six groups of rats containing 6 rats each were treated as follows: (i) ND – the control group was given a normal diet; (ii) high-fat-diet (HFD) – the control group included HFD rats that were given no treatment; (iii) HFD+lovastatin – the standard treatment group consisting of HFD rats that were given lovastatin, brand name "Cutcholand", Hygeia Pharmaceuticals, Islamabad, Pakistan, at a dose of 10 mg/kg body weight (bw) daily; (iv) HFD+CCFE – comprised six HFD rats that were given CCFE at a dose of 20 mg/kg bw daily; (v) HFD+CaONPs – comprised six HFD rats that were given CaONPs at a dose of 2 mg/kg bw daily; (vi) HFD+SynS – comprised HFD rats that were given a synergistic solution of CCFE (10 mg/kg) and CaONPs (1 mg/kg bw) daily. As the HFD+SynS group of rats were given a synergistic solution of CCFE and CaONPs, it included half the doses of both groups CCFE and CaONPs. Lovastatin, CCFE, CaONPs, and SynS were administered orally.

Induction of hyperlipidemia

Albino male Wistar rats (*Rattus norvegicus*) weighing 180 to 200 g were purchased from Bio Labs Pvt. Ltd., Islamabad, Pakistan. For induction of hyperlipidemia, rats (n=30) were given a HFD, according to [26] for 4 weeks. Normal control rats (n=6) were not given a HFD. The HFD included 81.7% rat feed (HiTech, Lahore, Pakistan), 16% fat (Biolab Zrt, Budapest, Hungary), 2% cholesterol (Biolab Zrt, Budapest, Hungary), and 0.3% deoxycholic acid (Merck, Darmstadt, Germany).

Measurement of body weight

The bw of 3 rats randomly selected from each group was measured every Monday using an electric balance.

Collection of blood and calculation of the atherogenic index of plasma (AIP)

After 8 weeks of treatment, blood was collected after anesthetizing the rats. A single intramuscular injection of ketamine hydrochloride (JHP Pharmaceuticals, LLC, Rochester, USA) at 1 mg/kg bw was used. Blood samples were collected from tail veins in sterile vials. Blood samples were used to collect plasma by centrifugation at 11×g for 15 min in a SIGMA® 1-14 centrifuge (Taufkirchen, Germany). The plasma was examined for high-density lipoprotein cholesterol (HDL-c), triglycerides (TG), alanine transaminase (ALT), alkaline phosphatase (ALP), and aspartate transaminase (AST) using commercial kits purchased from Pearl Scientific Instruments, Rawalpindi, Pakistan. To analyze HDL-c and TG, an automatic apparatus (MICROLAB-300-CAT-10 ELITech, Sées, France) was used. An automatic apparatus COBAS

Table 1. The major bioactive constituents of CCFE identified by GC-MS

RT	Name of Compound	Area %	CAS#	MF	MW
11.276	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	2.28	28564-83-2	$C_6H_8O_4$	144.12
15.779	Phenol, 4-(ethoxymethyl)-	10.45	57726-26-8	$C_9H_{12}O_2$	152.19
17.475	2,6-Di-tert-butyl-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	1.27	10396-80-2	$C_{15}H_{24}O_2$	236.35
17.762	Cyclododecane	1.62	294-62-2	$C_{12}H_{24}$	168.32
18.283	Butylated Hydroxytoluene	1.08	128-37-0	$C_{15}H_{24}O$	220.35
20.454	Ethyl α-d-glucopyranoside	28.91	34625-23-5	$C_8H_{16}O_6$	208.21
21.836	Carbonic acid, eicosyl vinyl ester	1.31	2243791-78-6	$C_{23}H_{44}O_3$	368.6
25.456	Palmitic acid	3.92	57-10-3	$C_{16}H_{32}O_2$	256.42
27.884	Oleic acid	4.35	112-80-1	$C_{18}H_{34}O_2$	282.5
28.162	Octadecanoic acid	1.91	57-11-4	$C_{18}H_{34}O_2$	282.5
30.960	Hexanedioic acid, bis(2-ethylhexyl) ester	6.93	103-23-1	$C_{22}H_{42}O_4$	370.6
32.595	Bis(2-ethylhexyl) phthalate	8.77	117-81-7	$C_{24}H_{38}O_4$	390.6

RT – retention time; CAS# – chemical abstracts services registry number; MF – molecular formula; MW – molecular weight (g/mol).

INTEGRA-400-Plus (Roche Diagnostics Ltd., Basel, Switzerland) was used to analyze ALT, ALP, and AST. For the calculation of AIP, the Dobiasova and Frohlich [27] formula was used as follows:

$$\mathrm{AIP} = \log\left(\frac{\mathrm{TG}}{\mathrm{HDLc}}\right)$$

Histopathological analysis of gastric mucosa and liver

The da Silva et al. [28] method was followed to analyze gastric mucosa and liver with a few modifications. As the CCFE, CaONPs, and SynS were orally administered, we studied the histopathology of the gastric mucosa to assess the direct toxic effects of different treatments on rat gastric ulceration. The organs of the rats were separated, cleaned, and placed in an automatic tissue processor (Tissue-Tek VIP-5, Sakura, Japan) for impregnation and cleaning after slaughter. For dehydration, specimens were placed in a tissue processor overnight. The specimens were embedded in paraffin blocks with a tissue embedding station (Tissue-Tek TEC-5, Sakura, Japan) before being cut into serial sections. Tissue sections 5 μm thick were cut using a rotary microtome (BIOBASE, Beijing, China). Hematoxylin and eosin (Merck, Darmstadt, Germany) were used to stain the tissue samples after collection. Finally, the stained tissue fragments were observed under a light microscope (L101 Hinotek, Beijing, China) at 40× magnification.

Statistical analysis

The arithmetic mean, standard deviation, and Duncan's multi-range test (DMRT) were analyzed by the statistical package for sSocial Sciences (SPSS 16.0, New York, USA). All the results are expressed as the mean and standard deviation (mean±SD). For the comparison of group means, one-way analysis of variance (ANOVA) was carried out. If there was a significant difference in group means, then DMRT was carried out.

RESULTS

Characterization of *C. colocynthis* fruit extracts (CCFE)

Gas chromatography-mass spectroscopy (GC-MS) was performed to characterize the volatile compounds. The total ion chromatogram is shown in Supplementary Fig. S1. The most abundant compounds with the highest peak area percentages were ethyl α-d-glucopyranoside (28.91), 4-(ethoxymethyl)-phenol (10.45), Bis(2-ethylhexyl) phthalate (8.77), and hexanedioic acid, bis(2-ethylhexyl) ester (6.93). Fatty acids included oleic acid (4.35), palmitic acid (3.92), octadecanoic acid (1.91), and carbonic acid (1.31) (Table 1). The total phenolic content of CCFE was found to be 203.52±9.24 mg/g of gallic acid equivalent. The total flavonoid content of CCFE was 173.56±11.05 mg/g of quercetin equivalent.

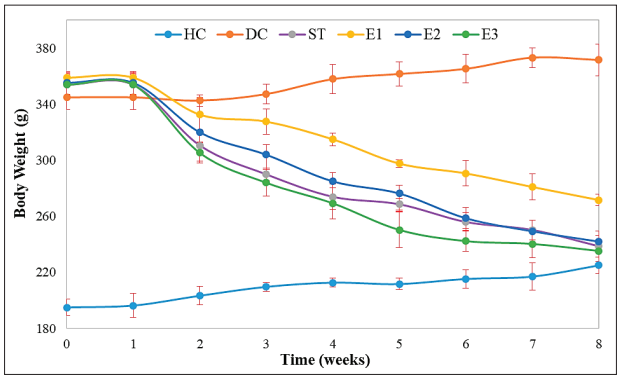

Fig. 1. The effect of CCFE, CaONPs, and SynS on the body weight of rats. Data are expressed as the mean±SD (n=3). HFD – high-fat-diet-fed (HFD) rats with no treatment; HFD+lovastatin – standard treatment group of HFD rats treated with lovastatin (10 mg/kg bw); HFD+CCFE – experimental group of HFD rats treated with CCFE (20 mg/kg bw); HFD+CaONPs – experimental group of HFD rats treated with CaONPs (2 mg/kg bw); HFD+SynS – experimental group of HFD rats treated with SynS, CCFE (10 mg/kg)+CaONPs (1 mg/kg).

The details of the linearity and validation of the protocol are given in Supplementary Table S1.

Characterization of calcium oxide nanoparticles (CaONPs)

We have previously published all the details of the characterization of CaONPs [29]. Briefly, the size of CaONPs was 35.93±2.54 nm. The absorbance peak (λ_{max}=325) indicated that the surface of the nanoparticles contained phenolics and flavonoids from CCFE. The XRD pattern elucidated the cubic crystalline structure. The vibrations of FTIR indicated adsorption of phenols (3639 cm^{-1}), alkanes (2860 cm^{-1}), alkynes (2487 cm^{-1}), amines (1625 cm^{-1}) and carboxylic acids (1434 cm^{-1}) (Supplementary Fig. S2). The green synthesized CaONPs revealed considerable stability with varying salinity, time, and pH. The dialysis membrane *in vitro* release indicated excellent bioavailability of CaONPs over 10 h [29].

Effect of CCFE, CaONPs, and SynS on body weight

After the induction of hyperlipidemia, the body weight of rats was measured every Monday during the eight weeks of the experiment. CCFE, CaONPs, and SynS significantly reduced the body weight of HFD rats. The decrease in body weight was consistent with time (Fig. 1). There was no significant difference between the results of the HFD+lovastatin rats and HFD+CaONPs rats. The decrease in body weight of HFD+SynS rats was significantly more significant than that of the HFD+lovastatin rats.

Table 2. Effect of CCFE, CaONPs, and SynS on the atherogenic index of plasma (AIP)

Treatment	TG	HDL-c	AIP = log (TG/HDL)
ND	$100.27\pm1.04_a$	$77.84\pm0.47_d$	$0.11\pm0.00_a$
HFD	$216.23\pm2.55_d$	$37.39\pm0.56_a$	$0.76\pm0.01_e$
HFD + LOVASTATIN	$111.50\pm0.54_b$	$76.58\pm1.06_d$	$0.16\pm0.01_c$
HFD + CCFE	$122.80\pm1.73_c$	$68.72\pm2.15_b$	$0.25\pm0.01_d$
HFD + CaONPs	$101.88\pm2.55_a$	$70.66\pm1.49_c$	$0.16\pm0.02_c$
HFD + SynS	98.98±0.94a	$75.79\pm1.22_d$	$0.12\pm0.01_b$

Data are expressed as the mean±SD (n = 3). Different alphabets in subscript in a column indicate significant differences in group means calculated by DMRT. TG – triglycerides; HDL-c – high-density lipoproteins-cholesterol; ND – healthy rats given only normal diet; HFD – high-fat-diet-fed (HFD) rats with no treatment; HFD + lovastatin – standard treatment group of HFD rats treated with lovastatin at 10 mg/kg bw; HFD + CCFE – experimental group of HFD rats treated with CCFE at 20 mg/kg bw; HFD + CaONPs – experimental group of HFD rats treated with CaONPs at 2 mg/kg bw; HFD + SynS – experimental group of HFD rats treated with SynS (CCFE at 10 mg/kg + CaONPs 1 mg/kg).

Effect of CCFE, CaONPs, and SynS on the atherogenic index of plasma (AIP)

The AIP is an important measure of cardiovascular health. The AIP of HFD rats was significantly higher, indicating a significant risk of cardiovascular disorders, whereas treatment groups treated with lovastatin, CCFE, CaONPs, and SynS exhibited significantly lower risk of cardiovascular disease as per the AIP values; there was no significant difference between HFD+lovastatin rats and HFD+CaONPs rats; however, HFD+SynS rats showed a significant decrease in AIP that was almost comparable to the healthy control rats (Table 2).

Effect of CCFE, CaONPs, and SynS on some biochemical parameters of liver function

Some biochemical parameters of liver function were studied at the end of the 8-week treatment. All treatment-group rats treated with lovastatin, CCFE, CaONPs, and SynS showed an elevation in alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate transaminase (AST); the lowest increase in

Table 3. Effect of CCFE, CaONPs, and SynS on some of the parameters of liver function

Readings	ALP	ALT	AST
ND	$139.21\pm4.30_{a}$	$59.93\pm2.27_{a}$	$46.05\pm3.33_{a}$
HFD	$264.79\pm7.29_{f}$	$99.09\pm2.84_{e}$	$70.92\pm2.58_{f}$
HFD + lovastatin	$192.80\pm11.87_{d}$	$82.25\pm2.24_{c}$	$61.07\pm2.17_{d}$
HFD + CCFE	$199.33\pm8.29_{e}$	$92.67\pm3.50_{d}$	$65.83\pm4.03_{e}$
HFD + CaONPs	$176.88\pm6.82_{c}$	$82.10\pm2.65_{c}$	$54.49\pm2.28_{c}$
HFD + SynS	$153.29\pm6.77_{b}$	$71.76\pm3.72_{b}$	$51.89\pm3.03_{b}$

Data are expressed as the mean±SD (n = 3). Different alphabets in subscript in a column indicate significant differences in group means calculated by DMRT. ALP – alkaline phosphatase; ALT – alanine transaminase; AST – aspartate transaminase; ND – healthy rats given only normal diet; HFD – high-fat-diet-fed (HFD) rats with no treatment; HFD + lovastatin – standard treatment group of HFD rats treated with lovastatin at 10 mg/kg bw; HFD + CCFE – experimental group of HFD rats treated with CCFE at 20 mg/kg bw; HFD + CaONPs – experimental group of HFD rats treated with CaONPs at 2 mg/kg bw; HFD + SynS – experimental group of HFD rats treated with SynS (CCFE at 10 mg/kg + CaONPs 1 mg/kg).

ALP, ALT, and AST was recorded in the HFD+SynS rat group (Table 3).

Effect of CCFE, CaONPs, and SynS on the histopathology of the stomach and liver

To assess the toxicity of CCFE, CaONPs, and SynS on gastric mucosa and hepatic tissue, histopathological analysis was performed. The histological micrographs of the different treatment groups are presented in Figs. 2 and 3. All treatment groups displayed almost no toxic effects on the gastric mucosa. The gastric mucosa and the submucosa showed no impairment at the end of the eight weeks of treatment. However, histological analysis of liver tissue revealed that the polygonal structure of hepatocytes was slightly disturbed by the treatments, and while lovastatin caused severe hepatotoxicity, CCFE, CaONPs, and SynS showed fewer hepatotoxic effects.

DISCUSSION

Citrullus colocynthis has been used to lower hyperlipidemia and control obesity. Previously, we conducted an ethnobotanical survey in the study area and found that *C. colocynthis* is used to treat obesity and gastritis [30]. In another study, we reported that CaONPs synthesized from CCFE fabricated with bioactive phenolic compounds and flavonoids showed significant bioactivity,

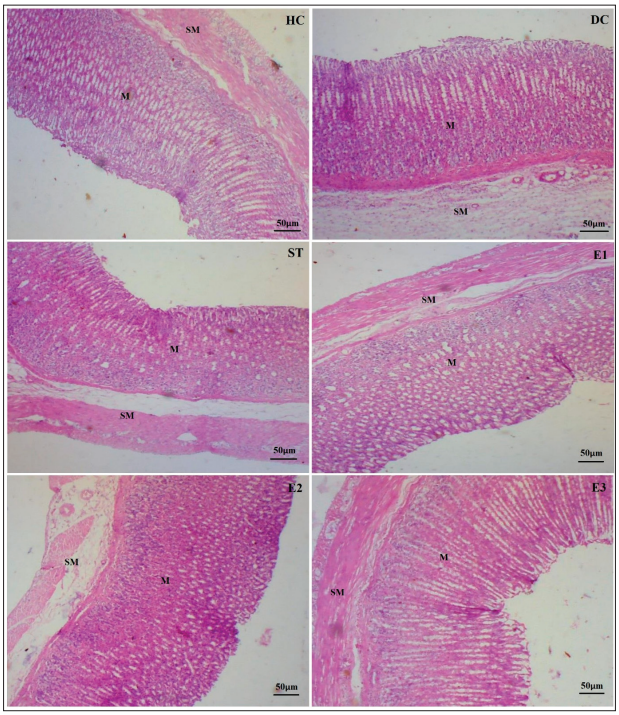

Fig. 2. Histological analysis of gastric mucosa of rats. ND – Healthy control group of healthy rats with no treatment; HFD – high-fat-diet-fed (HFD) rats with no treatment; HFD+lovastatin – standard treatment group of HFD rats treated with lovastatin (10 mg/kg bw); HFD+CCFE – experimental group of HFD rats treated with CCFE (20 mg/kg bw); HFD+CaONPs – experimental group of HFD rats treated with CaONPs (2 mg/kg bw); HFD+SynS – experimental group of HFD rats treated with SynS, CCFE (10 mg/kg)+CaONPs (1 mg/kg). M – mucosa; SM – submucosa; H&E 40×.

stability, bioavailability, and very low toxicity [29]. Herein, we studied the effect of CaONPs and their synergistic solution with CCFE on body weight, the atherogenic index of plasma, and the histopathology of the liver and stomach of rats.

Obesity is correlated with numerous metabolic and cardiovascular disorders [31], and maintaining the body weight within a certain limit is recommended for patients with chronic diseases [32]. In the present research, we studied the effects of different treatments on HFD hyperlipidemic rats. The results were compared with the standard antilipidemic drug lovastatin. A significant decrease in weight of HFD rats was recorded in rats that were administered lovastatin, CCFE, CaONPs, and SynS as compared to the HFD rats that were given no treatment. The weight-lowering potential of green synthesized CaONPs was comparable to that of lovastatin; however, SynS showed a higher weight-lowering potential as compared to lovastatin as it kept the body

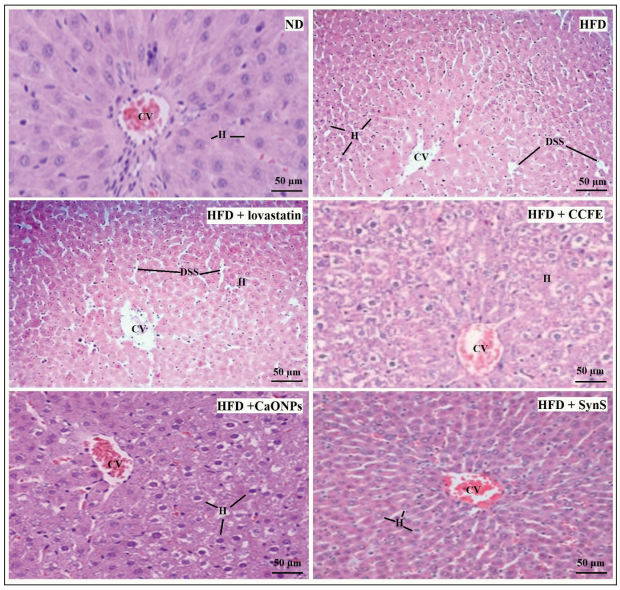

Fig. 3. Histological analysis of rats' liver. ND – Healthy control group of healthy rats with no treatment; HFD – high-fat-diet-fed (HFD) rats with no treatment; HFD+lovastatin – standard treatment group of HFD rats treated with lovastatin (10 mg/kg bw); HFD+CCFE – experimental group of HFD rats treated with CCFE (20 mg/kg bw); HFD+CaONPs – experimental group of HFD rats treated with CaONPs (2 mg/kg bw); HFD+SynS – experimental group of HFD rats treated with SynS, CCFE (10 mg/kg)+CaONPs (1 mg/kg). M – mucosa; SM – submucosa; (H&E 40×); CV – central vein; H – hepatocytes; DSS – dilated sinusoidal spaces; H&E 40×.

weight of rats near to that of the healthy control rats that were fed a normal diet and given no treatment. Previously, it was reported that different extracts of *C. colocynthis* possess a significant weight-lowering potential [33,34]. The decrease in body weight caused by CCFE could be attributed to phytoconstituents, including fatty acids and phenolics, especially oleic acid, which is well-known for its propensity to cause weight loss. Previously, it was documented that linoleic and oleic acids cause weight loss by decreasing the digestion and absorption of fats [35]. CaONPs and SynS caused more significant decreases in body weight as compared to CCFE, which could be due to the ability of nanoparticles to carry active compounds into the system by crossing membrane barriers. It was previously reported that nanoparticles fabricated with phytochemicals exhibit greater bioavailability and hyperlipidemic activity [14-16]. The results of this study are important because CaONPs and SynS can maintain body weight near normal values in HFD rats, which is necessary for controlling chronic disease-related complications.

Triglycerides (TG) and high-density lipoprotein cholesterol (HDL-c) are important indicators for the assessment of atherosclerosis and other cardiovascular impairments [36]. Disturbances in TG and HDL-c concentrations lead to persistent angina and other cardiovascular diseases that may prove fatal [37]. We examined the effect of CCFE, CaONPs, and SynS on blood lipid concentrations. The results of the study were compared with those of the standard antilipidemic drug lovastatin. We found that treatment with CCFE, CaONPs, and SynS led to significantly lowered TG concentrations and increased HDL-c concentrations in HFD rats. No significant difference was noted between the TG and HDL-c concentrations of healthy control rats and HFD rats treated with CaONPs and SynS. The decrease in TG concentrations shown by CaONPs and SynS in HFD rats was significantly greater as compared to lovastatin. Literature has also documented similar antilipidemic results shown by different parts of *C. colocynthis*. Seeds could significantly lower triglycerides and cholesterol concentrations [38], and fruit extracts reduced 87% of phospholipids and triglycerides [39]. The antilipidemic potential of CCFE may be attributed to the presence of phenolics, terpenoids, and flavonoids. It was established that phenolics and flavonoids have great antilipidemic potential [40]. Green synthesized CaONPs from CCFE showed better antilipidemic potential as compared to CCFE. The results of our study suggest that CaONPs can be potent antilipidemic carrier agents that can improve the antilipidemic activity of CCFE.

The atherogenic index of plasma (AIP) is an important indicator for assessing cardiovascular health [37,41]. A value of AIP≤0.11 is considered good, with a low risk of cardiovascular disorders; a value of AIP in the range of 0.12 to 0.21 indicates an intermediate risk of cardiovascular diseases, and a value of AIP≥0.22 is considered alarming, with a high risk of cardiovascular disorders [42,43]. In this study, we measured the AIP of HFD rats treated with CCFE, CaONPs, and SynS, and the results were compared with lovastatin. The AIP values calculated for the rats treated with CCFE, CaONPs, and SynS were significantly lower as compared to HFD rats with no treatment, indicating that CCFE, CaONPs, and SynS can reduce the risk of cardiovascular disorders in HFD rats. SynS showed the greatest improvement in cardiovascular health as per the AIP value of 0.12. The AIP values of HFD rats

treated with CaONPs and lovastatin were not significantly different. We found that SynS could manage the AIP of HFD rats near that of healthy rats. The results of this study suggest that the administration of SynS is more potent for hyperlipidemia and can reduce the risk of cardiovascular disorders. No study has reported the AIP values of green synthesized CaONPs or *C. colocynthis* extracts.

Nanoparticles are very toxic due to their highly reactive nature; however, CaO nanoparticles are less toxic than other metallic nanoparticles [44]. Our previous study found that CaONPs synthesized using CCFE were highly stable over time and to varying degrees in different pH and salinity [29]. Herein, we studied the biochemical parameters of liver function and the histology of the liver and stomach of HFD rats treated with CaONPs to assess their toxicity. Our results showed that all treatments, including lovastatin, CCFE, CaONPs, and SynS, were toxic to the liver, as revealed by changed biochemical parameters. However, lovastatin was more toxic than CaONPs and SynS. We found that SynS showed the lowest elevation in ALP, ALT, and AST among all treatment groups. The histological analysis of the liver revealed that the hepatocytes were severely disturbed by lovastatin, and CCFE, CaONPs, and SynS caused fewer disturbances. The histology of the gastric mucosa exhibited no impairment in all treatment groups. The mucosa and submucosa of gastric tissue were intact, showing no ulceration or toxic effects. Our previous study demonstrated that CaONPs are not toxic to macrophages and possess significant stability over time [29]. The present research has also documented that CaONPs that were green synthesized from CCFE were not toxic to the gastric mucosa and were comparatively less toxic to liver physiology and histology.

CONCLUSION

Based on the results of this study, it can be concluded that CCFE has a significant concentration of phenolics and flavonoids that stabilize the CaONPs. CaONPs, in combination with CCFE, enhance the weight-lowering and antilipidemic potential of CCFE. As per the estimated AIP values, a synergistic solution of CCFE and CaONPs can maintain a lower cardiovascular impairment risk in hyperlipidemic rats. CaONPs that are green synthesized from CCFE are less toxic to the gastric mucosa and less toxic to the liver's histology and physiology than lovastatin.

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Conflict of interest disclosure: The authors declare no conflicts of interest.

Data availability statement: All data underlying the reported findings have been provided as part of the submitted article and are available at: https://www.serbiosoc.org.rs/NewUploads/Uploads/Mazher%20et%20al_Dataset.pdf

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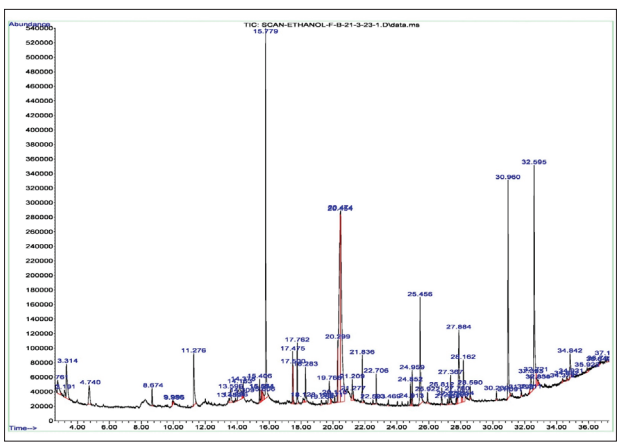
SUPPLEMENTARY MATERIAL

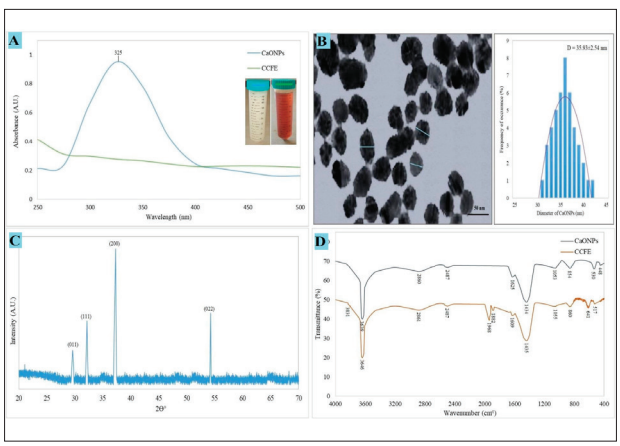
Supplementary Table S1. Method validation, total phenolic, and total flavonoid contents in *C. colocynthis* fruit extracts

Standard	Linearity range (µg/mL)	Calibration curve	R^2	LOD (mg/g)	LOQ (mg/g)	Concentration present in CCPE (mg/g)
Gallic acid	125-1000	y=0.0005x-0.001	0.9999	1.51	5.033	203.52±9.24 GAE phenolics
Quercetin	125-1000	y=0.0008x-0.0004	1	2.615	8.718	173.56±11.05 QE flavonoids

LOD – limit of detection; LOQ – limit of quantification; GAE – Gallic acid equivalent; QE – Quercetin equivalent.



Supplementary Fig. S1. Total ion chromatogram (TIC) of CCFE.



Supplementary Fig. S2. Characterization of CaONPs. A – UV-Vis spectrograph of CaONPs; B – TEM micrograph of CaONPs; C – XRD spectrograph of CaONPs; D – FTIR spectrograph of CaONPs.