ANALYSIS

54(275), November 1, 2018

ISSN 2278–5469 EISSN 2278–5450

Discovery

Characterizing and evaluating the potential of turmeric to attenuate hypercholesterolemia through animal modeling

Aniza Ishaque[1]✉, Moazzam Rafiq[1], Ashfa Ghani[1], Anum Sabir[1], Eman Manzoor[1], Sara Arshad[1], Hafiz Saad Bin Mustafa[2]

Hypercholesterolemia is the chief risk aspect for causing coronary heart ailments. Food has significant potential in managing this life threatening condition. Turmeric and its bioactive component, curcumin have therapeutic effect against hypercholesterolemia. In this study turmeric and its extract were evaluated for its hypocholesterolemic effect in high cholesterol induced rats for thirty days. For this purpose, extract of turmeric powder were assessed for total phenolic, total flavonoid and its antioxidant potential via screening tests like DPPH and FRAP assay. At the end of the study turmeric extract(conventional solvent extraction) and turmeric extract (supercritical fluid extraction) administered rats were kept fasted overnight and then they were analyzed for their serum lipid profile including HDL, LDL and triglycerides and total cholesterol and obtained data was subjected to statistical analysis. According to the analysis TPC (743.10 ± 8.19mg GAE/100g), TFC (75.14± 4.12 mg/g), DPPH (65.10± 1.03%) and FRAP (191.61 ± 4.1 uMFe^{2+}/g) showed their maximum values at 90% methanolic extract in conventional solvent extraction and Supercritical Fluid Extraction showed its maximum value at 4000psi regarding pressure factor. HDL(36.733 ± 2.195 mg/dL), LDL (74.825 ± 3.905 mg/dL), TG(74.825 ± 3.905 mg/dL) and TC (218.94 ± 13.345 mg/dL) showed decreased values at thirtieth day of study period as compared to start of study, showing positive effects in supercritical extract fed rats. Showing turmeric extract has beneficial effects in reducing cholesterol values.

INTRODUCTION

Phytonutrients are naturally-arising chemicals found in the plants and the plant-based foods, for instance in beans, fruits, grains and vegetables. The dietary treatment grounded on phytonutrients have strengthened nutrition that does not only aims at improving the general health, moreover it helps at easing the medial cost thereby safeguarding the financial benefits (Epstein *et al*., 2010). Likewise, some foods increases the immunity in the body and enhance the amount of antioxidants in the body which forms a protecting wall in the body and save body from the dangerous effects (Prasad *et al*., 2014). There are few spices which have amazing effect on the body due to their foremost quality that is presence of phytoceutics this quality plays as a protective agent in the body (Srinivasan, 2005). The popular Indian spice known as "turmeric" is a perennial rhizomatous sage belonging to the family Zingiberaceae also popular as Curcuma Longa. Pakistan is the second leading producer of turmeric but yield is pitifully less. In several areas, Kasur is greater than eighty percent of whole turmeric fabrication. It also has property of coloring agent and food preservative in China, India and South East Asia. As a home therapy it has been used in traditional remedy for numerous diseases including cough, diabetic wounds, biliary disorders, anorexia, hepatic disorders, sinusitis and rheumatism. A lot of work has been done to determine the pharmacological actions and biological value of turmeric and its extract (Chattopadhyay *et al*., 2004).

Spice turmeric's chemical composition constitute of protein (6.3%), minerals (3.5%), moisture (13.1%), protein (6.3%) fat (5.1%) and carbohydrates (69.4%). In turmeric powder the essential oil is about (5.8%) in turmeric there is cineol (1%), borneol (0.5%), a-phellandrene (1%), cineol (1%), zingiberene (25%), sabinene (0.6%) and sesquiterpine (53%). Turmeric's active component "curcumin" also known as diferuloylmethane (3–4%) give yellow color to turmeric and consist of curcumin I (94%), and curcumin III (0.3%) curcumin II (6%). In 1815 for the first time, curcumin was separated. Roughly determined its chemical structure first and then Whiting in 1973, Whiting determined its melting point at around 176–177°C; forming a brownish and reddish saline along with alkali is solvable intoacetic acid, ethanol, ketone and chloroform (Chattopadhyay *et al*., 2004). The non-toxicity, safety and tolerability of curcumin at a dose about 8g/day is revealed to be safe in numerous human studies. Moreover, FDA has declared turmeric and 'curcumin', the bioactive component of turmeric as GRAS (generally regarded as safe), (Kumar *et al*., 2016).

[1]National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan; [2]Directorate of Oilseeds, Ayub Agricultural Research Institute, Faisalabad, Pakistan;
✉*Corresponding author:* National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan; Email: anizarana499@gmail.com

The nutraceutical compound "curcuminoids" are universally used for the food preparations as well as medicine. The best extractants for the turmeric extraction are alcohols and acetate leading to high yields (Popuri and Pagala, 2013). Turmeric's yellow bioactive part which is called curcumin, which has looks asan extensive array in bioticactions, comprising of anti-oxidant, anti-coagulant, anti-inflammatory, anti-mutagenic, anticarcinogenic, antidiabetic, antifertility, antivenom, antibacterial, antifungal, antiulcer, antiprotozoal, antiviral, antifibrotic, hypotensive and hypocholesterolemic actions (Chattopadhyay *et al*., 2004). Turmeric powder, turmeric's derivatives and curcumin and countless new extracts were exists from rhizomes assumed as a biological property. The ether extract of the Curcuma longa have been found to show hypolipidemic effect and lowered fatty acids, cholesterol and triglycerides in humans (Pungcharoenkul *et al.,* 2011). Hypercholesterolemia is defined as high levels of cholesterol in the blood also known as dyslipidemia, hypercholesterolemia may be dietary or it may be familial hypercholesterolemia (FH), FH clinical manifestations has constantly been shown to be linked with premature death and increased coronary heart disease (Austin *et al*., 2004). FH is the type of hyperlipidemia and it is transferred automatically to the offspring's and it have more chances in those children whose parents are suffering to this disease. Heart disease is eminent at birth due to previous atherosclerosis. FHTG is automatically transferring disease it is complemented due to obesity, high sugar level diseases, high blood pressure, high uric level, abdominal pain, dyspnea, eruptive xanthoma, lipemiaretinalis and memory loss are the major symptoms developed in patients with marked hypertriglyceridemia which is >1000 mg/dL (Shaikh and Kamal, 2012).

In Pakistan, heart attack causes approximately 23.7-26.9% & 30% losses in women and men correspondingly of the overall cardiovascular events. Elevated LDL-cholesterol (low density lipoprotein) levels in the blood increases the risk of getting heart ailments while healthy HDL-cholesterol (high density lipoprotein) levels decreases the chances of getting heart disease (Shaikh and Kamal, 2012).

Turmeric exhibit ten folds higher anti-oxidative capacity owing to presence of the curcumin, because curcumin is lipophilic in nature, on reaction with oxygen free radicals. It becomes phenyl radical which can be further restored on reaction with water soluble antioxidants (Ak and Gulcin, 2008). Additionally, it can activate superoxide dismutase and glutathione thereby strongly quenching hydroxyl, nitrogen dioxide and singlet oxygen free radicals (Kunwar and Priyadarsini, 2011).

According to several studies report hypolipidemic effect of curcumin and its claim to work by lowering serum cholesterol and triglycerides level in rats fed hyperlipidemic diet for inducing hyperlipidemia rats. Moreover, it also increases fecal cholesterol excretion thereby prompting alterations in 7-a-hydroxylase's gene expression, enzyme leading to homeostasis of the cholesterol. Likewise, the action of the rate limiting enzyme, HMG-CoA reductaseworking for the cholesterol biosynthesis likewise modified by curcumin induction (Qinna *et al*., 2012).

By assessing the all of the overhead mentioned details, the recent research was done to assess valuable influence of the curcumin on dyslipidemia. Curcumin was extracted from turmeric by conventional method and fed orally to study subjects. However, evaluation of bioactive property and antioxidative potential of its extract is the limelight of this study. The effect of turmeric extracts against hypercholesterolemia.

MATERIALS AND METHODS

Obtaining raw material

The randomized controlled trial was being conducted in Faculty of Food Nutrition and Home Sciences, National Institute of Food Science and Technology, University of Agriculture Faisalabad. Turmeric was procured from residential marketplace.

Preparation of sample

First the turmeric rhizome was washed and then dried in the hot air cabinet dryer at 60°C for 8-10 hours. This dried turmeric rhizome were ground by using grinder to obtain a fine powder. It was stored at ambient temperature for further analysis.

Extraction Analysis

The extract of turmeric powder will be prepared by using solvent methanol at different concentrations by following the method already mentioned by Abdelbaky*et al.* (2012).

Preparation of turmeric extract

By using methanol at three different concentrations and by following the protocol of Bagchi *et al*. (2012), turmeric extract will be prepared.

Treatments	Solvent
T1	Methanol 50%
T2	Methanol 70%
T3	Methanol 90%

Supercritical Fluid Extraction

For isolation of curcumin, time dependent SC-CO2 extraction will be conducted at 3500, 4000 and 4500psi under optimized conditions of temperature and time set at 150min to accelerate solvation and mass transfer as mentioned by Wakte *et al* (2011).

Treatments	Pressure
T1	3500 psi
T2	4000 psi
T3	4500 psi

Phytochemical screening tests

Total phenolic contents

Using Folin-Ciocalteu method, turmeric powder was accessed for its total phenolic content as defined by Ebrahimzadeh *et al.*, 2008a on basis of formation of phosphotungstic blue reduced from phosphotungstic acid, resulting in increase in absorbance owing to increase in number of such phenolic groups which are aromatic. Concerning this drive, extract of volume 50 µL was individually taken in a test tube containing 750 µL of 20% sodium carbonate solution, 250µL of Folin-Ciocalte's reagent and with distilled water, volume was made up to 5mL. Against control, by using UV/visible light Spectrophotometer (CECIL CE7200), after two hours that besides sample extract had all the reacting reagents. Overall polyphenols was assessed and the resultant tenets were expressed as equivalent of gallic acid (mg gallic acid/100g).

For calculating each of the extract's total phenolic compounds in equivalents of gallic acid (GAE), the used formula was as:

$$C = V/m \times c$$

C = Whole phenol substances (milligram/gram of plant extract, in gallic acid equivalents)

Total Flavonoid Contents

By applying the method of Nabavi*et al*. (2012), spectrophotometric machine was applied to conclude the total flavonoid ratio of each extract which work on the base of flavonoid-aluminium complex development. The standard used for measuring total flavonoids in turmeric powder extracts was Quercetin. In a volumetric flask of 10ml, 1ml of extract was added and with the addition of 5ml of distilled water, volume raised to 5 mL trailed by accumulation of 0.3 mille Liter sodium nitrite having 5% (weight/volume). Subsequently 5 minute interval, from 10% (w/v) $AlCl_3$, 0.6mL was taken in addition 2 mL of 1M NaOH was assorted at 6 minute, and this was trailed by the accumulation of distilled water of volume 2.1 mL. At 510 nm using UV/visible light spectrophotometer, the absorbance was measured. Resultant data was stated in mg per 100 gram of extract as quercetin equivalents.

In vitro experimentation

Free radical sequestering capability

By using method of Ebrahimzadeh *et al*, (2008b) DPPH radical scavenging activity of turmeric powder extracts was accessed for its DPPH radical foraging property To define DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical hunting action of turmeric powder extracts, sample extract of 0.025 mL was dissolved in 10 mL of corresponding solvent for preparing sample solution. 3 milliliter of newly formed solution of DPPH in relevant solvent (6×10-5Mole) was miscellaneous with 77 µL tester extract. For about 15 minutes, each sample was set aside in dark place at a room heat and absorbance fall was dignified at about 517 nanometer going on the Ultraviolet/visible light spectrophotometer. Likewise, absorbing capacity of absolute tester having the volume alike of solvent and of DPPH solution excluding extract of turmeric was set and at the similar wavelength absorbance was assessed on UV/visible light spectrophotometer. The unrestricted radical-foraging action turmeric powder extracts were obtainable in the proportion fall in DPPH owing to the known volume of every extract.

$$Absorbance\ decline(\%) = 100 \times \{\frac{AB - AA}{AB}\}$$

AB = Blank sample's absorbance when time = 0 min.
AA = Tried extract solution's absorbance when time't' = 15 minutes

(FRAP) Ferric reducing antioxidant power assess

The reducing influence in turmeric powder extracts stood by quantifying ability of removes by reduction of ferric tripyridyltriazine into ferrous i.e. having blue color the detection of which was made at 593 nano meter as defined by Berker*et al.* (2007). Preparation of reagent of FRAP include mingling of 2.5 mL TPTZ (10 milliMole), 25 mille Liter of buffer of acetate (0.1 M at pH 3.6) in addition 2.5 milliliter ferric chloride (20 milliMole) which was kept warm for 10 minutes at 30° Centigrade. For accessing reducing influence of turmeric extract proximately 1.5 mL of reagent of FRAP had been assorted with 100 micro Liter of turmeric powder extract or regular and distilled water of volume 100 µL. Then on UV/visible light spectrophotometer at 593nm, absorbance was seen. By means of trolox (0-500 µmol/mL), a calibration curve was drawn and it was stated as equivalent of µmoltrolox per gram of trial.

Selection of Best Treatment

On the basis of anti-oxidant assay and extraction efficiency one best treatment will be selected for further analysis from turmeric powder extracts.

In Vivo Studies

Sixteen Sprague Dawley male rats were acquired from National Institute of Health (NIH), Islamabad and accommodated in the National Institute of Food Science and Technology's Animal room, Agriculture Uni. Faisalabad, Pakistan. Throughout the experimental trial, relative humidity (55±5%) and temperature (23±2°C) of the Animal Room was organized with 12 hours dark-light retro turn by turn. When study was initiated, few rats slaughtered for acquiring each study's baseline values. Rats were divided into four groups, group1 was kept as control and was given standard diet, 2nd group nurtured on elevated cholesterol diet throughout the study & kept such as positive control, 3rd group was also given high cholesterol was treated with curcumin extracted by conventional extraction and likewise group 4 rats were fed on high cholesterol food was cured with curcumin extracted by supercritical fluid extraction.

Feed & drink intake was noted on daily basis, during the whole study period whereas body weight was measured on the weekly basis. Towards the end of the study, rats were kept fasted for a night and then sacrificed. Blood samples of rats were taken in tubes coated with EDTA through cardiac puncture for purpose of hematological examining and to quantify lipid profile altitudes in serum of the blood was collected in non-coated tubes through Rifah lab, Faisalabad, Pakistan.

Treatments used in the study

*G0	Negative Control	Normal diet
*G1	Positive control	High cholesterol diet
*G2	Turmeric extract fed *(CSE)	High Cholesterol Diet with turmeric extract(CSE)
*G3	Turmeric extract fed *(SFE)	High Cholesterol Diet with turmeric extract(SFE)

*CSE=Conventional Solvent Extraction
*SFE=Supercritical Fluid Extraction
*G0=Normal rats
*G1=Hypercholesterolemic rats
*G2=Hypercholesterolemic rats
*G3=Hypercholesterolemic rats

Drink and feed intake

Total intake of feed for each of rat group was quantified by subtracting fallen feed from the total given diet on daily basis throughout the study (Wolf and Weidbrode, 2003). Likewise, daily water intake was documented alongside for every group by observing the graduated bottle's variances.

Gain in Body weight

Throughout the efficacy trial body weight was accessed on weekly basis to determine change in body weight by effect of two diets with different type of extract given in rats feed.

Lipid profile of serum

Lipid profile in serum includes triglycerides, cholesterol, low density lipoprotein and high density lipoproteins were calculated owing to their corresponding procedures, which is detailed as follows:

Triglycerides

In all serum testers, total triglycerides was measured by using liquefied triglycerides (GPO–PAP) process given by Demonty (2010).

Cholesterol

Cholesterol level in serum was calculated by applying CHOD–PAP technique by using the decorum of Kim *et al*. (2011).

Low density lipoprotein

Testers of blood serum were also determined on behalf of low density lipoproteins (LDL) by using the technique of Alshatwi *et al.* (2010).

High density lipoprotein

Sample of the serum was examined for HDL by HDL Cholesterol Precipitant manner like given by Alshatwi *et al.* (2010).

Statistical Examination

Resultant records for every factor were imperiled to statistic study for defining level of significance (Montgomery, 2008). Using Factorial Design, analysis of variance was calculated although means were deduced by Latin Square Design.

RESULTS AND DISCUSSION

***In-vitro* antioxidant activity for Conventional and Supercritical Fluid Extract**

Mean antioxidant potential (Table 1) regarding methanol solvent at three different concentrations (50%, 70% and 90%) have shown maximum antioxidant potential at 90% (65.10± 1.03) followed by 70% (47.403 ± 1.22) and 50% (36.677 ±1.13) correspondingly. Mean antioxidant potential (Table 2) regarding three pressures (3500psi, 4000psi and 4500psi) in Supercritical fluid extraction have shown maximum antioxidant potential at 4000psi (62.760 ±2.31) followed by 4500psi (54.007 ±2.11) and 3500psi (41.573±2.40).

Ramkumar and Rajasankar, 2016 has determined antioxidant activity of methanolic extracts of turmeric powder. Results interpretations have declared that maximum oxidation inhibition (60.510 ± 1.03%) was recorded at 90%, followed by 70% (47.403 ± 1.22%) and 50% (36.677 ±1.13%). In the previous studies, Tilak *et al*. (2004) sedated 52% free toxins foraging capability of methanolic extract of turmeric by 1,1-diphenyl-2-picrylhydrazyl assay. As previously reported by Gulcin and Ak (2008) that curcumin has robust antioxidant capacity against DPPH as 62.20 %.

Ferric Reducing Antioxidant Potential

The (table 3) shows antioxidant potential for treatment at at the three different percentages for methanol and the trend depicts highest antioxidant potential at 90% (191.61 ± 4.1 uMFe^{2+}/g) followed by 70% methanolic extract (175.06 ± 3.81 uMFe^{2+}/g) and methanol 50% (146.65 ± 3.47 uMFe^{2+}/g) or ferric reducing antioxidant potential (FRAP) assay. FRAP assay (Table 4) regarding three pressures (4500psi, 4000psi and 3500psi) show high antioxidant potential at 4000psi (191.00 ± 3.01 uMFe^{2+}/g) followed by 4500psi (178.97 ± 4.61 uMFe^{2+}/g) and 3500psi (152.06 ± 2.23 uMFe^{2+}/g).

Selection of best treatment

Best treatment will be selected on the basis of their phenolic content and antioxidant potential, therefore from CSE best treatment selected is 90% methanolic extract and from SFE best treatment selected is 4000psi extract. And these are given to groups G2 and G3 respectively while G1 is positive control with induced hypercholesterolemia and G0 kept as negative control.

Efficacy study

Present research study was done to compare health promoting benefits of turmeric extracts collected by two means conventional solvent extraction and supercritical fluid extraction in the management of hypercholesterolemia. Regarding this aspect, animal study was conducted on male Sprague Dawley rats because of release in controlling, diminutive fear for dangerous health possessions of established ingredient and lenience in enquiry of every constraint as compared to the human study as it was not possible to search out such human volunteers who can trust on such treatment for a long period of time. Rat study was classified into four groups 2 kept as control 1 positive control (on high cholesterol diet) and 1 negative control (on normal diet), the other 2 groups were given extract 1 group fed CSE and 1 group fed SFE orally 2ml/rat/day for 30 days duration. At start of study few rats were surrendered to achieve baseline values. Feed and drink intake was measured daily while body weight on weekly basis. At the end of the study collected sera was tested for hematological parameters including HDL, TG, TC and LDL.

Feed Intake

Mean squares in relation to feed intake showed prominent differences with respect to weeks and experimental groups (Table 5). Maximum feed intake was recorded in group G3 26.5±0.66 g/rat/day fed on supercritical extract followed by group G1 26.39± 0.84 g/rat/day kept as positive control followed by G2 25.9± 0.89 g/rat/day and G0 24.75± 0.59 g/rat/day rats kept negative control. As per week trend feed intake was seen increasing with maximum feed intake recorded at week 4 26.52 ±0.83 g/rat/day and minimum at week 1 25.17± 0.70 g/rat/day while in week 3 and week 4 results depict constant feed intake 26.41± 0.70 g/rat/day.

Water Intake

Mean squares for drink intake was effected significantly due to treatments as well as weeks (Table 19). Maximum drink intake (Table 6) was observed in G4 fed supercritical extract (23.14 ± 0.73) ml/rat/day, followed with G2 (22± 0.60ml/rat/day) and G1 (21.7 ± 0.38 ml/rat/day) with minimum intake in G0 (20.38± 1.49 ml/rat/day) kept as negative control. Similar trend was observed for weeks (Table 6) with increase in water intake with every increasing week, displaying maximum water intake in week 4 (23.16 ± 0.48 ml/rat/day), followed by week 3 (22.38± 1.01 ml/rat/day), week 2 (21.53±0.9 ml/rat/day) and week 1 (20.1 ± 0.80 ml/rat/day) with least value of drink intake.

Body weight

It is seen that mean values of body weight was significantly lower in CSE group (222.70± 6.87 g) and SFE group (218.91± 4.80 g) as compared to the positive control group fed high cholesterol diet (338.81 ± 7.41 g) followed by negative control group kept as normal control (253.2± 5.25 g).

Table 1 Table for mean of CSE

Treatment	Mean
T1 (50%)	36.677 ±1.13 c
T2 (70%)	47.403 ± 1.22 b
T3 (90%)	60.510 ± 1.03 a

Table 2 Means of DPPH(%) in SFE of turmeric

Treatment	Mean
T1 (3500psi)	41.573±2.40 c
T2 (4000psi)	62.760 ±2.31 a
T3 (4500psi)	54.007 ±2.11 b

Table 3 Means for FRAP uMFe^{2+}/g of CSE of turmeric

Treatment	Mean
T1 (50%)	146.65 ± 3.47 c
T2 (70%)	175.06 ± 3.81 b
T3 (90%)	191.61 ± 4.1 a

Table 4 Means for FRAP uMFe^{2+}/g of SFE of turmeric

Treatment	Mean
T1 (3500psi)	152.06 ± 2.23c
T2 (4000psi)	191.00 ± 3.01 a
T3 (4500psi)	178.97 ± 4.61b

Table 5 Means for feed intake

Groups	Weeks				Mean
	W1	W2	W3	W4	
G0	23.87 ± 0.28	24.62±0.75	25.08± 0.83	25.43± 0.50	24.75± 0.59
G1	24.07± 0.80	26.73± 0.92	27.06 ±0.78	27.71± 0.86	26.39± 0.84
G2	26.01± 0.90	24.81 ±0.57	26.64± 1.03	26.23± 1.07	25.9± 0.89
G3	26.73±0.84	27.23±0.55	26.88±0.39	25.22± 0.89	26.5±0.66
Mean	25.17± 0.70	26.41± 0.70	26.41± 0.75	26.52 ±0.83	

Table 6 Means of weeks and groups interaction

Groups	Weeks				Mean
	W1	W2	W3	W4	
G0	19.33± 1.78	20.42± 2.37	21.23± 1.56	20.54± 0.26	20.38± 1.49
G1	20.88± 0.10	21.68± 0.31	22.01± 0.53	22.25 ± 0.59	21.7 ± 0.38
G2	19.81± 0.20	21.24± 0.59	22.49± 1.18	24.46 ± 0.45	22± 0.60
G3	20.55± 1.15	22.79± 0.36	23.8± 0.79	25.42± 0.64	23.14 ± 0.73
Mean	20.1 ± 0.80	21.53± 0.9	22.38± 1.01	23.16 ± 0.48	

Table 7 Means of weeks and groups interaction

Groups	Weeks				Mean
	W1	W2	W3	W4	
G0	229.51±4.56	235.73± 4.47	255.02± 5.45	292.54± 6.55	253.2± 5.25
G1	235.57± 5.56	307.13± 7.77	397.16± 8.85	415.39± 7.47	338.81 ± 7.41
G2	235.07± 5.24	228.83± 4.82	220.32± 6.31	206.61 ± 9.75	222.70± 6.87
G3	234.17± 6.62	222.03± 4.25	217.67± 5.21	201.78± 3.15	218.91± 4.80
Mean	233.58 ± 5.49	248.43± 5.32	272.54± 6.45	279.08 ± 6.73	

Table 8 Analysis of variance for High Density Lipoprotein (milligram/deciliter)

Source of variation	Degree of Freedom	Sum of Squares	Mean Squares	F-value
Treatments	3	1030.86	343.620	23.72 **
Days	1	73.89	73.886	5.10 *
Treatment x days	3	49.86	16.621	1.15 ^{NS}
Error	16	231.79	14.487	
Total	23	1386.40		

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01)

Table 9 Table for treatments means of HDL(mg/dL)

Treatment	Mean
G0	48.185 ± 5.435 a
G1	30.918 ± 3.466 b
G2	37.733 ± 2.195 bc
G3	38.092 ± 2.859 c

Table 10 Means for effect of days on HDL (mg/dL)

Days	Mean
0	35.977± 3.17 b
30	39.977 ± 3.80 a

'a' is showing greater significance as compared to 'b'

Table 11 Table for treatments and days interaction mean of HDL (mg/dL)

Parameters	Days		Mean
Treatment	**0**	**30**	
G0	47.87 ± 5.421	48.50 ± 5.45	48.185 ± 5.435
G1	29.80 ± 3.790	32.03 ± 3.142	30.915 ± 3.466
G2	32.29 ± 2.424	35.17 ± 1.967	33.73 ± 2.195
G3	33.94 ± 1.055	42.24 ± 4.664	36.71 ± 2.859
Mean	35.97 ± 3.17	39.48 ± 3.80	

Table 12 AOV table for total cholesterol (mg/dL)

Source of variation	Degree of Freedom	Sum of Squares	Mean Square	F-value
Days	1	2514.1	2514.13	16.71 **
Treatment	3	9757.7	3252.56	21.62 **
Days x treatment	3	532.2	177.41	1.18 ^{NS}

Error	16	2407.5	150.47
Total	23	15211.5	

Table 13 Means for treatment and days interaction for total cholesterol (mg/dL)

Parameters	**Days**		
Treatment	**0**	**30**	**Mean**
G0	191.21± 10.33	177.89± 10.120	184.55± 10.225
G1	245.01± 11.910	235.98 ± 10.23	240.51 ± 11.12
G2	236.32 ± 14.910	206.45 ± 12.99	221.38 ± 13.95
G3	233.77± 12.67	204.11 ± 14.02	218.94 ± 13.345
Mean	226.57 ± 12.455	206.10 ± 11.84	

Table 14 Table for treatment mean of total cholesterol

Treatment	**Means**
G0	184.55 ± 10.225
G1	240.50 ± 11.07
G2	221.39 ± 13.345
G3	218.94 ± 13.95

Table 15 Table for treatment means of triglycerides (mg/dL)

Treatment	**Mean**
G0	84.44± 4.65 b
G1	119.19± 4.64 a
G2	75.82 ± 3.905c
G3	75.83 ± 4.015 c

Table 16 Means of days in triglycerides (mg/dL)

Days	**Mean**
0	92.185± 4.655 a
30	85.478± 4.34 b

Table 17 Table for treatments and days interaction means of LDL (mg/dL)

Parameters	**Days**		**Mean**
Treatment	0	30	
G0	117 ± 10.31	103.37 ± 11.69	110.18± 11
G1	174.56± 11.23	151.20 ± 10.13	162.88 ± 10.68
G2	163.83 ± 11.08	112.63 ± 10.39	138.23 ± 10.73
G3	160.34 ± 10.42	110.46 ± 13.91	135.4 ± 12.16
Mean	153.93 ± 10.76	119.41 ± 11.53	

Table 18 Table for treatment means of LDL (mg/dL)

Treatment	**Mean**
G0	121.42± 11 a
G1	162.88± 10.68 b
G2	132.72± 10.73 b
G3	128.10± 12.16 b

Hematological analysis

The present research study was assessed in hypercholesterolemic and normal rats to determine effect of curcumin on HDL, triglycerides, total cholesterol and LDL.

High Density Lipoprotein (HDL)

The "good cholesterol" slogan is credited to HDL because of HDL's ability to Reverse Transport of cholesterol by removing circulating cholesterol from tissues and arteries back to the liver. Predominantly, this takes place where deposition of cholesterol is in form of atheroma in the sub-endothelial space. Moreover, because LDL has role in taking toxic cholesterol from liver into the circulating blood it is called as 'bad cholesterol'. Presently, major devotion is given towards reduction of LDL by various nutrition implements however, countless epidemiological research studies have expanded role of the HDL toward the cardiovascular health organization (McEneny *et al*., 2013).

Polyphenols seek major attention in the régime of diet based rehabilitation, core attention has been given to polyphenols as the heart protective representative. Among polyphenols, curcumin is given emphasis in restraining this threat. The means squares for effect of diet (Table 8) show highly significant effect of diet on HDL cholesterol levels while effect of days has depicted significant effect, however the interaction between treatments and days shows non-significant effect on HDL cholesterol levels in 30 days trial. The table 9 shows highest HDL values in G0 group (48.185 ± 5.435), followed by G3 (38.092 ± 2.859), G2 (37.733 ± 2.195) and G1 (30.918 ± 3.466). The days (Table 9) have significant impact on HDL cholesterol, showing improved HDL values at 30 (39.977 ± 3.80) day as compared to the 0 day (35.977± 3.17).

The table 10 depicts effect of different extracts on HDL values at 0 and 30 days, showing no change in HDL value in rats on normal diet i.e. G0 (negative control) which increase from 47.87 ± 5.421 to 48.50 ± 5.45 mg/dL, G1 rats (positive control) shows insignificant change in HDL which at 0 day 29.80 ± 3.790 changes to 32.03 ±3.142 mg/dL. In group G2 cholesterol value change from 32.29 ± 2.424 to 35.17 ± 1.967 mg/dL for conventional solvent extract and in G3 HDL values increased from 33.94 ± 1.055 to 42.24 ± 4.664mg/dL for SFE group. Results depict significant effect of SFE on HDL levels as compared to CSE group.

Mean squares (Table 11) for effect of treatment and days have shown highly significant and significant results respectively, while the interaction between treatment and days is non-significant. Treatment means for HDL (Table 9) shows highest values for HDL in negative control group 48.185 ± 5.435mg/dL followed by SFE group 38.092 ± 2.859 mg/dL than CSE group 37.733 ± 2.195 mg/dL and positive control group 30.918 ± 3.466 mg/dL.

Total cholesterol

Mean squares (Table 12) for effect of treatment and days have shown highly significant results, while the interaction between treatment and days is non-significant. Treatment means for total cholesterol (Table 14) shows highest values for total cholesterol in G1 240.50 ± 11.07 mg/dL followed by CSE group 240.50 ± 11.07 mg/dL than SFE group 218.94 ± 13.95 mg/dL and positive control group 184.55 ± 10.225 mg/dL. Results from (Table 13) depicts that SFE have greater impact in deceasing total cholesterol as compares to CSE fed rats. Further, SFE have greater effect in reducing total cholesterol 204.11 ± 14.02 mg/dL as compared to CSE 206.45 ± 12.99 mg/dL.

Triglycerides

The table 15 shows highest triglycerides values in rats fed on high cholesterol diet119.185 ± 4.465 mg/dL. Mean value of triglycerides show lowest values of triglycerides in SFE 74.825 ± 3.905 mg/dL as compared to CSE 75.825 ± 4.645 mg/dL. Table 16 have depicted that triglyceride value declined to 85.478± 4.34 mg/dL on 30 days as compared to 0 days which was higher 92.185± 4.655 mg/dL. Trend shows that with days triglycerides values have fallen. In the present study, by providing curcumin based diet to the hypercholesterolemic rats, positive effects were seen to accomplish lipid related anomalies by subduing the elevated LDL level. Convincingly, turmeric polyphenols augmented edibles are effective to amend numerous lifestyle related maladies.

Low Density Lipoprotein

The table 15 shows highest LDL values in rats fed on high cholesterol diet 162.88 ± 10.68 mg/dL. Mean value of triglycerides show lowest values of triglycerides in SFE 135.4 ± 12.16 mg/dL as compared to CSE 138.23 ± 10.73 mg/dL. Table 17 have depicted that triglyceride value declined to 115.95± 11.53 mg/dL on 30 days as compared to 0 days which was higher 156.61± 10.76 mg/dL. Trend shows that with days LDL values have fallen.

CONCLUSION

On whole, trial concerning hypercholesterolemic perception have discovered that equally conventional solvent extract plus supercritical fluid extract act beneficial in normalizing plasma lipid profile, however, supercritical fluid extract proved to be more effective in providing curative tool for cardiovascular disorders arising due to high circulating cholesterol levels in the blood.

REFERENCES

1. Abdelbaky, M.S., F.E. Hanaa and K.M.A. Nedaa. 2009. Effect of some citrus peels on hypercholesterolemic rats. The 1st International and 4th Arab Annual Scientific Conference on: Academic Accreditation for Higher Specific Education Institutions and Programs in Egypt and Arab World "Reality and Expectation". 1626-1639.
2. Ak, T. and I. Gulcin. 2008. Antioxidant and radical scavenging properties of curcumin. Chem. Biol. Interact. 1: 27-37.
3. Alshatwi, A.A., M.A. Al-Obaaid, S.A. Al-Sedairy, A.H. Al-Assaf, J.J. Zhang and K.Y. Lei. 2010. Tomato powder is more protective than lycopene supplement against lipid peroxidation in rats. Journal of Nutrition Research. 30:66-73.
4. Austin, M.A and S.E. Humphries. 2004. Familial hypercholesterolemia and coronary heart disease: A huge association review. American Journal of Epidemiology. 160:420-429.
5. Bagehi, A. 2012. Extraction of curcumin. J. Enviro. Sci. Toxical. Food Technol. 1(3):1-16
6. Chattopadhyay, I., K. Biswas., U. Bandyopadhyay and R.K. Banerjee. 2004. Turmeric and curcumin: Biological actions and medicinal applications. Current Science. 87:44-53.
7. Demonty I, Lin Y, Zebregs YE, Vermeer MA, van der Knaap HC and Jäkel M. 2010. The citrus flavonoids hesperidin and naringin do not affect serum cholesterol in moderately hypercholesterolemic men and women. Nutrition. 140:1615-1620.
8. Epstein, J., I.R. Sanderson and T.T. MacDonald. 2010. Curcumin as a therapeutic agent: The evidence from in vitro, animal and human studies. Br. J. Nutr. 103:1545-1557.
9. Kim, J.I., J.K. Paik, O.Y. Kim, H.W. Park, J.H. Lee, Y. Jang and J.H. Lee. 2011. Effects of lycopene supplementation on oxidative stress and markers of endothelial function in healthy men. Atherosclerosis. 215:189-195.

10. Kumar, A., M. Singh, P. P. Singh, S.K. Singh, P. Raj, K. D. Pandey. 2016. Antioxidant efficacy and curcumin content of turmeric (*curcuma-longa* l.) Flower. International Journal of Current Pharmaceutical Research. 8: 112-114.
11. Kunwar A. and K.I. priyadarsini. 2011. Free radicals, oxidative stress and importance of antioxidants in human health. J. Med. Allied Sci. 53-60.
12. McEneny, J., L. Wade, I.S. Young, L. Masson, G. Duthie, A. McGinty, C. McMaster and F. Thies. 2013. Lycopene intervention rteduces inflammation and improves HDL functionality in moderately overweight middle-aged individuals. The J. Nutr. Biochem. 1: 163-168.
13. Montgomery, D.C. 2008. Design and Analysis of Experiment. 7th Ed. John Wiley and sons. Inc. Hoboken, NJ, USA. 16: 162-264.
14. Nabavi, S.M., S.F. Nabavi, A.H. Moghaddam, W.N. Setzer and M. Mirzaei. 2012. Effect of silymarin on sodium fluoride-induced toxicity and oxidative stress in rat cardiac tissues. An. Brazilian Acad. Sci. 84(4) :1121-1126.
15. Popuri, A.K. and B. Pagala. 2013. Extraction of curcumin from turmeric roots. Int. J. Innov. Res. Stud. 2:289-299.
16. Prasad, S., S.C. Gupta, A.K. Tyagi and B.B. Aggarwal. 2014. Curcumin, a componenr of golden spice: From bedside to bench and back. Biotechnol. Adv. 6: 1053-1064.
17. Pungcharoenkul, K. and P. Thongnopnua. 2011. Effect of different curcuminoid supplement dosages on total in vivo antioxidant capacity and cholesterol levels of healthy human subjects. Phytotherapy Research. 25:1721-1726.
18. Qinna, N. A., B.S. Kamona, T.M. Alhussainy, H.Taha, A.A. Badwan and K.Z. Matalka. 2012. Effects of prickly peared dry leaves, artichoke leaves, turmeric and garlic extracts, and their combinations on preventing dyslipidemia in rats. ISRN Pharm. 12: 1-7.
19. Shaikh, Q. and A.K. Kamal. 2012. Journal of the Pakistan Medical Association. 6: 623-632.
20. Srinivasan, K. 2005. Plant foods in the management of diabetes mellitus: Spices as beneficial antidiabetic food adjuncts. Int. J. Food Sci. Nut. 6: 399-414.
21. Wakte, P.s., B.S. Sachin, A.A. Patil, D.M. Mohato, T.H. Band and D.B. Shinde. 2011. Optimization of micriwavw, ultrasonic and supercritical carbon dioxide assisted extraction techniques for curcumin from Curcuma longa. Separ. Sci. Technol. 79:50-55.
22. Wolf, B.W. and S.E. Weisbrode. 2003. Safety evaluation of an extract from Salaciaoblonga. Food Chemistry. Toxicology. 41:867-874.

Article Keywords
Turmeric, hypercholesterolemia, cholesterol, antioxidant

Article History
Received: 30 July 2018
Accepted: 26 September 2018
Published: 1 November 2018

Citation
Aniza Ishaque, Moazzam Rafiq, Ashfa Ghani, Anum Sabir, Eman Manzoor, Sara Arshad, Hafiz Saad Bin Mustafa. Characterizing and evaluating the potential of turmeric to attenuate hypercholesterolemia through animal modeling. *Discovery*, 2018, 54(275), 423-431