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EVALUATION AND PHOTOCHEMICAL SCREENING OF ANTIHYPERLIPIDEMIC ACTIVITY OF TERMINALIA ARJUNA

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Abstract:

The present study was undertaken to evaluate the phytochemical constituents, antihyperlipidemic activity, and antioxidant potential of the ethanolic extract of Terminalia arjuna in high-fat diet (HFD)-induced hyperlipidemic rats. Preliminary phytochemical screening of the extract revealed the presence of flavonoids, tannins, phenolic compounds, saponins, glycosides, and triterpenoids, which are known for their therapeutic properties. Acute toxicity studies performed according to OECD guidelines indicated that the extract was safe at the tested dose levels.

Hyperlipidemia was induced in Wistar albino rats by administration of a high-fat diet for the experimental period. The animals were divided into five groups: normal control, hyperlipidemic control, standard drug-treated group, and two test groups receiving Terminalia arjuna ethanolic extract at doses of 200 mg/kg and 400 mg/kg respectively. Atorvastatin (10 mg/kg) was used as the standard drug. Various biochemical parameters including body weight, total cholesterol (TC), triglycerides (TG), HDL, LDL, VLDL, creatinine, urea, uric acid, AST, ALT, and ALP were evaluated. Antioxidant parameters such as superoxide dismutase (SOD), catalase, reduced glutathione (GSH), and lipid peroxidation (MDA) were also estimated.

The hyperlipidemic control group showed significant increases in body weight, serum lipid profile, liver enzyme markers, renal parameters, and lipid peroxidation levels, along with decreased HDL and antioxidant enzyme levels. Treatment with Terminalia arjuna extract significantly reduced TC, TG, LDL, VLDL, AST, ALT, ALP, creatinine, urea, uric acid, and MDA levels while increasing HDL, SOD, catalase, and GSH levels when compared with the hyperlipidemic control group. The high-dose extract (400 mg/kg) demonstrated greater pharmacological activity and produced results comparable to atorvastatin.

the study indicate that Terminalia arjuna possesses significant antihyperlipidemic and antioxidant activities, which may be attributed to the presence of bioactive phytoconstituents. Thus, the plant extract may serve as a potential natural therapeutic agent for the management of hyperlipidemia and associated oxidative stress.

Keywords: *Hyperlipidemia, Terminalia arjuna, Antihyperlipidemic activity, Antioxidant activity, High-fat diet, Lipid profile, Flavonoids, Oxidative stress, Atorvastatin, Phytochemical screening.*

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INTRODUCTION:

India has seen an era of prosperity in ancient times especially in the use of traditional knowledge in therapeutics. The use of "Gou-mutra" (cow urine), Swarna Bhasma and medicinal herbs are just a few to mention when we boast of India's richness in the ancient medical panorama. Our country has always been in a privileged position, sitting on a gold mine of well-recorded and well-practiced knowledge of traditional medicine in the form of the Vedas and folklore practice among the different tribes [1]. The process and assimilation continue and today traditional medical practices are obliged to accommodate to the norms of modern biomedicine. However, there is growing awareness among the scientific community and the general public about the intrinsic value of traditional medicine and as a result Ayurveda, Unani and Siddha have entered the mainstream to complement biomedicine. The challenge is to integrate the best of the different healing traditions to meet the healthcare needs of society [2].

India sits in a privileged position in the use of traditional health resources as it covers world's biodiversity hotspot in the North eastern states (Indo- Burma hot spot) and the Western Ghats. But, in contrast to this, developments made by India in the field of pharmaceutics are significantly less as compared to countries that are at a lower privileged position in terms of the traditional knowledge base [3]. India has not been able to capitalize on this wealth by promoting its use in the developed world despite their renewed interest in traditional medicines. The forecast is that the global market for herbal products is expected to be $5 Trillion by 2050 [4]. Total global herbal market is of size 62.0 billion dollars, in this India's contribution is only one billion dollars [5].A major cause of this is the export of medicinal herbs to other countries at low revenue. However these herbs are processed by them after proper research and sold in the Indian market at a higher price. Owing to this, India is actually not able to make proper use of its wealth. It is now high time that India should start planning strategies to overcome this situation. This can be achieved by judicious product identification based on diseases found in the developed world for which no medicine or only palliative therapy is available; such herbal medicines will find speedy access into those countries. Backward integration from market demands will pay rich dividends. Strategically, India should enter through those plant-based medicines which are already well accepted in Europe, USA and Japan. Recently there has been a shift in universal trend from synthetic to herbal medicine, which is termed as 'Return to Nature' and our country definitely has a potential to make proper use of this situation owing to its resources [4].

In the present situation, it is apparent that whatever important the concerns and demands of the policy makers, health professionals and public may be, they do not, in any way, stops the increasing trend of using herbal medicines. As a result, herbal medicine-based Traditional Medicine (TM) practices remain widespread in developing countries and that of Complementary and Alternative Medicine (CAM) is increasing rapidly in developed countries [6]. This trend of growing and widespread use of herbal medicines is likely to increase even further throughout the world in the coming years with more and more scientific evidence of their quality, efficacy and safety coming from the researchers [7].

Traditional medicines will only be taken seriously and accepted by primary health care systems when experimental and clinical data establish an evidence base. The safety and effectiveness of the traditional medicines can be achieved by high standard studies. It is necessary to observe the co-medication of medicinal plants and biomedicine and the practitioners and clinicians are encouraged to watch out for adverse as well as their beneficial interactions. Clinical studies instead of focusing exclusively on efficacy could focus also on effectiveness of the herbal product. The changing global economic scene and changing paradigm in drug development might present an advantage in the attempt to officially register traditional medicines with governmental agencies [8].

Hyperglycemia, hyperlipidemia: risk factors of Metabolic Syndrome

Metabolic syndrome (MetS) identifies patients at increased risk of developing CVD and type 2 diabetes mellitus (T2DM). Throughout the world, consumption of highenergy diet together with a general reduction in the level of physical activity is causing an alarming health problem, namely MetS, a cluster of physiologically interconnected biochemical, clinical, and metabolic factors.Central obesity and insulin resistance are acknowledged as important causative factors,[9–11] together with other associated conditions, including physical inactivity, [12], ageing [13]and hormonal imbalance [14]. These factors increase the susceptibility of individuals to disorders like CVD, non-alcoholic fatty liver disease (NAFLD), T2DM and central obesity (Fig 1). These MetS related risk factors are also associated with the generation of degenerative reactive oxygen species (ROS) in the body [15].Previous studies predict that MetS would confer to a 5-fold increase in the risk of T2DM and a 2-fold increase in the risk of developing CVD over the next 5 to 10 years [16]. Also, International diabetes federation (IDF) estimated that around 20-25 percent of the world's adult population has

suffered from MetS, and they are twice likely to die from and three times as likely to have a heart attack or stroke compared with people without the syndrome.

Technological advances in Western countries have resulted in shifting of energy balance towards more sedentary lifestyle accompanied by increased calorie intake. MetS is a chronic disorder resulting from the imbalance between energy consumption and energy utilization. It is characterized by high blood pressure, impaired glucose tolerance, dyslipidemia, abdominal fat accumulation [17]. World Health Organization (WHO) predicted that about 350 million people worldwide would have diabetes and would be the 7th leading cause of death by 2030.

Epidemiological studies further reveal the worldwide prevalence of MetS. It is a consequence of changes in human dietary habit that comprise of high glycemic load carbohydrates like fructose and excessive detrimental saturated fatty acids (SFA) from animal sources. Excess consumption of SFA and fructose in the diet leads to disturbances in fatty acid (FA) and carbohydrate metabolism [18]. Clinical studies have confirmed that fructose can induce the features of MetS [19]. This kind of diet has less amount of beneficial polyunsaturated fatty acids (PUFA) [17]. Mutton Fat (MF), a widely used form of fat from an animal source is reported to contain more SFAs (68%) than PUFAs [20]. High-carbohydrate high-fat (HCHF) diet used for inducing MetS consist of SFA rich MF and fructose. HCHF diet initiates the symptoms of MetS by generating oxidative stress (OS) and leads to changes in the ultra-structure of affected organs and in the level of essential trace elements(TE) in the body [21]. Imbalance of specific TE may change the normal glucose, insulin, and lipid metabolism and increase the blood pressure, owing to diabetes and cardiovascular complications [22].

MATERIALS AND METHODS:

Plant collection and authentication

terminalia arjuna. Used For This Study Were Collected from Ranga Reddy Dist., Telangana. of Brassica Nigra**.**was identified and authenticated by Dr. Madhava Chetty, Asst. Professor Sri Venkateshwara University, Tirupathi.

Experimental animals

Healthy adult Wistar albino rats (150–200 g, 8–10 weeks old) of either sex were used to evaluate the antihyperlipidemic activity and phytochemical relevance of *Terminalia arjuna*. The animals were procured from a CPCSEA-registered breeder and housed in polypropylene cages under standard laboratory conditions (22 ± 2 °C, 55 ± 10% humidity, 12/12 h light–dark cycle) with standard pellet diet and water ad libitum, and were acclimatized for seven days prior to experimentation. The study protocol was approved by the Institutional Animal Ethics Committee in accordance with guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Hyperlipidemia was induced by feeding a high-fat diet for 28 days. Animals were divided into five groups (n=6): normal control (standard diet + 0.5% CMC), hyperlipidemic control (HFD + vehicle), standard (HFD + atorvastatin 10 mg/kg, p.o.), and two test groups receiving ethanolic extract of *T. arjuna* at 200 and 400 mg/kg, p.o. respectively. Treatments were administered orally for 28 days, and parameters such as body weight, serum lipid profile (TC, TG, HDL, LDL, VLDL), atherogenic index, liver enzymes (AST, ALT), were evaluated to assess antihyperlipidemic activity.

Preparation of Terminalia arjuna Extract

50 grams of shade-dried, coarsely powdered bark of Terminalia arjuna and placed in a Soxhlet extractor. About 250 mL of ethanol was added to a 500 mL round-bottom flask, and the Soxhlet apparatus was assembled with a condenser ensuring continuous water circulation. The system was heated on a heating mantle to allow the solvent to boil, condense, and siphon repeatedly through the plant material. Extraction was continued for 6–8 hours After completion, the extract was allowed to cool and the solvent was filtered. The dried extract was weighed to calculate percentage yield and stored used for the experiment.

Acute Toxicity Studies [23]

Acute toxicity studies of Terminalia arjuna extract were carried out to determine its safety and toxic effects before evaluating its antihyperlipidemic activity. The study was performed according to OECD guideline 423 (Acute Oral Toxicity Method). Healthy Wistar albino rats were fasted overnight and administered the extract orally at different dose levels. After administration, the animals were observed continuously for the first 4 hours and periodically for 24 hours for signs of toxicity such as changes in skin, fur, eyes, salivation, tremors, convulsions, behavioral changes, respiratory distress, and mortality. Further observations were continued for 14 days to detect any delayed toxic effects. The extract did not produce mortality or significant toxic symptoms up to the tested dose level, indicating that the plant extract was safe for pharmacological evaluation. Based on the results, suitable doses were selected for the antihyperlipidemic study.

Experimental Animals:

Acute oral toxicity of the ethanolic extract of *Terminalia arjuna* bark was evaluated in Wistar

albino rats according to the OECD Guideline 423 (Acute Oral Toxicity – Acute Toxic Class Method). Healthy female rats (150–200 g) were fasted overnight with free access to water and randomly divided into groups (n=3 per step). The extract was administered orally at a starting dose of 300 mg/kg body weight; based on the absence of mortality, the dose was escalated to 2000 mg/kg. Animals were observed continuously for the first 4 hours, periodically during the first 24 hours, and daily thereafter for 14 days for signs of toxicity such as changes in skin, fur, eyes, mucous membranes, behavior, tremors, convulsions, salivation, diarrhoea, lethargy, sleep, coma, and mortality. Body weights were recorded on days 0, 7, and 14. At the end of the observation period, animals were examined for gross pathological changes. The absence of mortality or significant behavioral changes up to 2000 mg/kg indicated that the extract is relatively safe, and experimental doses (e.g., 200 and 400 mg/kg) were selected as fractions of the safe dose for pharmacological studies.

High-Fat Diet (HFD) Induced Hyperlipidemia [24]

Hyperlipidemia was induced in experimental Wistar albino rats by administering a high-fat diet (HFD) for a period of 21–28 days to evaluate the antihyperlipidemic activity of Terminalia arjuna extract. The high-fat diet consisted of cholesterol, cholic acid, coconut oil or lard mixed with standard pellet feed, which increased serum lipid levels such as total cholesterol, triglycerides, LDL, and VLDL while reducing HDL levels. Continuous consumption of the HFD caused abnormal lipid metabolism and accumulation of fats in the bloodstream, thereby producing experimental hyperlipidemia similar to that observed in humans. After confirmation of elevated lipid parameters, the animals were treated with *Terminalia arjuna* extract and standard antihyperlipidemic drugs to assess their lipid-lowering effects.

Experimental procedure:

Healthy Wistar albino rats (150–200 g) were acclimatized for seven days under standard laboratory conditions. Hyperlipidemia was induced by feeding a high-fat diet (HFD) for 28 days. The animals were randomly divided into five groups (n = 6): Group I received standard diet with 0.5% CMC (normal control), Group II received HFD with vehicle (hyperlipidemic control), Group III received HFD with atorvastatin (10 mg/kg, p.o.) as standard, and Groups IV and V received HFD along with ethanolic extract of *Terminalia arjuna* at doses of 200 and 400 mg/kg body weight, p.o., respectively. All treatments were administered orally once daily for 28 days. Body weight was recorded weekly throughout the study. At the end of the treatment period, animals were fasted overnight and anesthetized; blood samples were collected via retro-orbital puncture for estimation of serum lipid profile parameters including total cholesterol, triglycerides, HDL, LDL, and VLDL, along with liver enzymes (AST and ALT). After blood collection, the animals were sacrificed, and liver tissues were excised, washed with normal saline, and preserved in 10% formalin for histopathological examination. The results obtained were statistically analyzed to assess the antihyperlipidemic activity of the extract.

Animal Grouping:

Group I Normal Control Standard pellet diet + 0.5% CMC (1 mL/kg**,** p.o.)
Group II Hyperlipidemic Control High-fat diet (HFD) + 0.5% CMC (1 mL/kg, p.o.)
Group III Standard HFD + Atorvastatin (10 mg/kg, p.o.)
Group IV Test Low Dose HFD + T. arjuna ethanolic extract (200 mg/kg, p.o.)
Group V Test High Dose HFD + T. arjuna ethanolic extract (400 mg/kg, p.o.)

PROCEDURE

Rats were divided into five groups each group contain six animals. Group I serves normal which receives normal saline (5ml/kg b wt) for last 28 days orally; Group II serves as positive control which receives 5% cholesterol diet for 3 months continuously; Group III serves as standard which receives Rosavastatin (20mg/kg b wt; I.P route) for last 28 days and Group IV, Group V serves as test which receives Brassica Nigra at dose of 250 and 500 mg/kg b wt, through oral route throughout the study period and also receives 5% cholesterol diet for last 28 days. After the study period animals were sacrificed by collecting blood through cardiac puncture and collected blood was centrifuged and serum was collected and biochemical analysis were performed lipid profiles for serum levels TC, TG, HDL, LDL, VLDL, Urea, Creatinine, uric acid and antioxidant studies SOD, GSH and Catalase.

Biochemical estimation [25-26]

a) **Estimation of weight gain:**

During the experimental period, the high cholesterol diet consumed and weight gained by rats was recorded on 0th, 14th and 28th day of EENC treatment. The pre-weighed food pellets (approximately 30g) were placed inside the hopper of the cage. The food consumed by individual rat was quantified by weighing leftover food in the hopper.

b) Estimation of total cholesterol (TC): (CHOD-PAP Method)

The reagents kits intended for the in-vitro quantitative determination of cholesterol in serum/plasma.

Principle: - The cholesterol esters are hydrolysed by enzyme cholesterol esterase to give free cholesterol and fatty acid molicules. This free cholesterol gets oxidised in presence of cholesterol oxidase to liberate cholset 4en-3one and H2O2. Liberated H2O2 by this reaction combines with phenol and 4 amino antipyrine in presence of peroxidise to form red colourquinonimine complex, the intensity of which is measured at 505 nm. (490-530nm) it is directly proportional to the cholesterol conc. Present in sample.

General system parameters:-
Wave length : 505 nm (490-530nm)
Incubation : 5 min
Sample volume : 10µl
Reagent volume : 1.0ml
Standard concentration: 200mg/dl

Procedure:-
Bring all the reagents of assay to room temperature.

	Procedure for 1ml			**Procedure for 3ml**		
	Blank	**Standard**	**Test**	**Blank**	**Standard**	**Test**
Enzyme reagent	1ml	1ml	1ml	3ml	3ml	3ml
Standard		10µl			30µl	
Sample			10µl			30µl

Mix well and incubate for 5 min at room temperature. Mix well and measure the absorbance of standard and test against the reagent blank at 505 nm. (490- 530nm)

Calculation: - Total cholesterol mg/dl = Abs TC/Abs STD ×200

c) Estimation of triglycerides (TG):(GPO-Method)
Diagnostic kit was used for estimation of triglycerides, which followed end point colorimetry enzymatic test using glycerol-3-phosphate oxidase.

Principle:
The enzyme, lipoprotein lipase catalyzes hydrolysis of TGs to glycerol and FAs. Glycerol then is phosphorylated in an ATP - requiring reaction catalyzed by glycerophosphate. The formed glycerophosphate is oxidized to dihydroxyacetone and H2O2in a glycerophosphate oxidase (GPO) catalyzed reaction. H2O2then reacts with 4 -AAP and 4 - chlorophenol under the catalytic influence of peroxidase to form colouredquinoneimine complex, the intensity of which was measured at 505nm[103].

$$\text{Triglyceride} + 3H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{3 - Fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerokinase}} \text{Glycero - 3 - phosphate} + \text{ADP}$$

$$\text{Glycero - 3 - phosphate} + O_2 \xrightarrow{\text{Glycerophosphate oxydase}} \text{DHAP} + H_2O_2$$

Reagents used:

Sl. No.	**Reagent composition**	**Conc. in the final test mixed**
1	Pipes butter	50mmol/l
2	4-Chlorophenol	5mmol/l
3	Mg^{2+}	5 mmol/l
4	ATP	1 mmol/l
5	Lipase	≥ 5000 U/l
6	Peroxidase	≥ 1000 U/l
7	Glycerol Kinase	≥ 400 U/l
8	Glycerol - 3- phosphate oxidase	≥ 4000 U/l

Standard: The concentration of standard triglyceride used was 200mg/dl

Assay & Procedure: Fresh clear and haemolysed serum was used for the estimation.

Reaction parameters:

1	Reaction type	End point
2	Wave Length	505 nm
3	Optical length	1 cm
4	Temperature	37°C
5	Measurement	Against reagent blank

Summary of assay details:

Pipetted in to test tube	Blank	Standard	Test
Reagent	1000 µl	1000 µl	1000 µl
Standard		10 µl	
Sample			10 µl

The reaction mixtures were mixed well and incubated for 10 min at 37°C.

The absorbance of sample and standard were measured against reagent blank at 505 nm.

Calculations:-

$$\text{Serum triglycerides (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 100$$

d) Estimation of high-density lipoprotein cholesterol (HDL-C):

Diagnostic kit was used for estimation of HDL cholesterol, which followed Cholesterol oxidase / peroxidase (CHOD-POD) method.

Principle: HDL-C is measured in the supernatant after the precipitation of the lipoproteins including chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins, intermediate-density lipoproteins directly from serum polyanions like phosphotungstic acid and along with MgCl2are added to an aliquot of serum an immediate heavy precipitation is formed. The precipitate then is sedimented by centrifugation and HDL cholesterol is measured in the clear supernatant, which is estimated by enzymatic method as described earlier in estimation serum of TC[104].

Reaction parameters:

1	Reaction type	End point
2	Wavelength	505 nm
3	Optical path	1 cm
4	Temperature	37°C
5	Measurement	Against reagent blank

Preparation:-

Take 0.5 ml of serum /plasma in to glass tube. Add 50 µl precipitating reagent. Mix well, leave it at R.T. For 10 min. centrifuge at 3000 r.p.m. for 10 min. take the clean supernatant for HDL cholesterol estimation.

Procedure:-

	B	S	T
Enzyme reagent	1 ml	1 ml	1 ml
Standard		0.01 ml	
Supernatant sample			0.01 ml

Mix well and incubate for 5 min at 37°c. Measure the absorbance of HDL&std at 510 nm.

Calculations: - HDL cholesterol mg/dl = Abs TH/Abs STD×200

e) Estimation of creatinine:

Method: Jaffe's method

Principle: Creatinine reacts with alkaline picrate to produce an orange-yellow colour (the jaffe's reaction).The absorbance of orange-yellow coloured complex formation is directly proportional to the creatinine concentration and is measured photo metrically at 500-520 nm[105,106]

Reagents:

1. Picric acid reagent 100ml
2. Alkaline buffer reagent 100ml
3. Standard creatinine 10ml
4. Acid reagent 10ml

Procedure: Set the Auto-analyser instrument with parameters given along with the kit. Prepare the working. Standard, test solution as per the protocol. Mix well and read initial absorbance (A0) 20 seconds after mixing and final absorbance (A1) 80 seconds after mixing at 505 nm.

Reaction parameters:

Reaction type	Kinetic with standard
Wave length	520nm (green filter)
Incubation temperature	Room temperature
Incubation time	30 sec
Read time	60 sec
No. of readings	2

Linearity	20 mg%
Sample volume	100µL
Reagent volume	1µL
Cuvette	1cm light path

Procedure:

	Blank	**Standard**	**Sample**
Working reagent	1ml	1ml	1ml
Standard		0.1ml	
Sample			0.1ml

Mix well and allow it to stand for 5 mins. Read absorbance for absorbance for standard and sample against distilled water at 520nm against blank.

For test ΔAT= A1T-A0T
For standard ΔAS= A1S-A0S

$$\text{creatinine} = \frac{\Delta AS}{\Delta AT}$$

f) Estimation of urea: [27-28]

Principle:

Urease catalyses the conversion of urea to ammonia and carbon dioxide. The ammonia released reacts with a mixture of salicylate, hypochlorite and nitropruside to yield a blue-green coloured compound (indophenols). The intensity of colour produced is proportional to the concentration of urea in the sample and is measured photometrically at 570nm or with yellow filter[107].

Reagents:

Urease reagent 50ml
Enzyme concentrate 1 vial
Colour reagent 50ml
Urea standard 2ml

Reaction Parameters

Mode of reaction	End point
Wave length	570nm
Temperature	Room temperature
Standard concentration	40mg%
Linearity	300mg/%
Blank	Reagent
Incubation time	10min
Cuvette	1cm light path

Procedure

	Blank	**Standard**	**Sample**
Working reagent	1000µL	1000µL	1000µL
Standard		10µL	
Sample			10µL

Mix and incubate for 5minutes at room temperature. Read the absorbance of the standard and sample against reagent blank.

Calculations:

$$\text{Urea concentration mg} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times 40(\text{standard con.})$$

g) Estimation of uric acid:

Principle:

$$\text{Uric acid} + O_2 + 2H_2O \xrightarrow{\text{Urase}} \text{llantoine} + CO_2 + H_2O_2$$

$$2H_2O_2 + \text{ESPAS} + \text{4 - AAP} \longrightarrow \text{Purple quinoeimine} + 4H_2O_2$$

Reagents:

Enzyme reagent 50ml
Standard 2ml

Parameters

Mode of reaction	End point
Wave length	546nm
Reaction slope	Increasing
Temperature	Room temperature
Standard concentration	5mg%
Linearity	25mg/%
Blank	Reagent
Reagent volume	1ml
Incubation time	10min
Cuvette	1cm light path

Procedure

	Blank	**Standard**	**Sample**
Enzyme Reagent	1000µL	1000µL	1000µL
Standard		10µL	
Sample			10µL

Mix and incubate for 5minutes at room temperature. Read the absorbance of the standard and sample against reagent blank.

Calculations:

$$\text{Uric acid mg/dl} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times \text{Stdcon}$$

Antioxidant enzymes Estimation [29]

Estimation of superoxide dismutase (SOD)

Known amount of tissue was weighed and washed in ice cold saline and homonized in ice cold 0.1M TrisHcl buffer for estimations.

The assay of SOD was based on the ability of SOD to inhibit spontaneous oxidation of adrenaline to adrenochrome. 0.05 ml supernatant was added to 2.0 ml of carbonate buffer and 0.5 ml of 0.01mM EDTA solution. The reaction was initiated by addition of 0.5 ml of epinephrine and the autooxidation of adrenaline (3× 10-4 M) to adrenochrome at pH 10.2 was measured by following change in OD at 480 nm. The change in optical density every minute was measured at 480 nm against reagent blank. The results are expressed as units of SOD activity (mg/wet tissue). One unit of SOD activity induced approximately 50% inhibition of adrenaline.

Estimation of catalase activity (CAT)

Catalase activity was determined spectrophotometrically according to previously published method. Briefly, to 1.95ml of 10mM H_2O_2 in 60mM phosphate buffer (pH=7.0), 0.05ml of the liver homogenate was added and rate of degradation of H_2O_2 was followed at 240nm per min. Catalase content in terms of U/mg of protein was estimated from the rate of decomposition of H2O2 using the formula k=2.303/ Δt × log (A1/A2) s-1 (A unit of catalase is defined as the quantity which decomposes 1.0 µmole of H_2O_2 per min at pH=7.0 at 25oC, while H_2O_2 concentration falls from 10.3 to 9.2 mM).

Estimation of glutathione (GSH) [30-31]

The assay is based on the formation of a relatively stable yellow product when sulphydryl groups react with DTNB. Briefly, proteins were precipitated using 10% TCA, centrifuged and 0.5 ml of the supernatant was mixed with 0.2M phosphate buffer (pH 8.0) and 10mM DTNB. This mixture was incubated for 10 min and the absorbance was measured at 412nm against appropriate blanks. The glutathione content was calculated by using the standard plot under same experimental conditions. Plotting the absorbance against GSH concentration (0.005- 0.042 µmoles) gave the standard curve. A linear correlation coefficient (r2 = 0.999) was obtained.

Results

Percentage of yield

$$Percentage\ of\ yield = \frac{Weight\ of\ crude\ extract}{Weight\ of\ dried\ material} \times 100$$

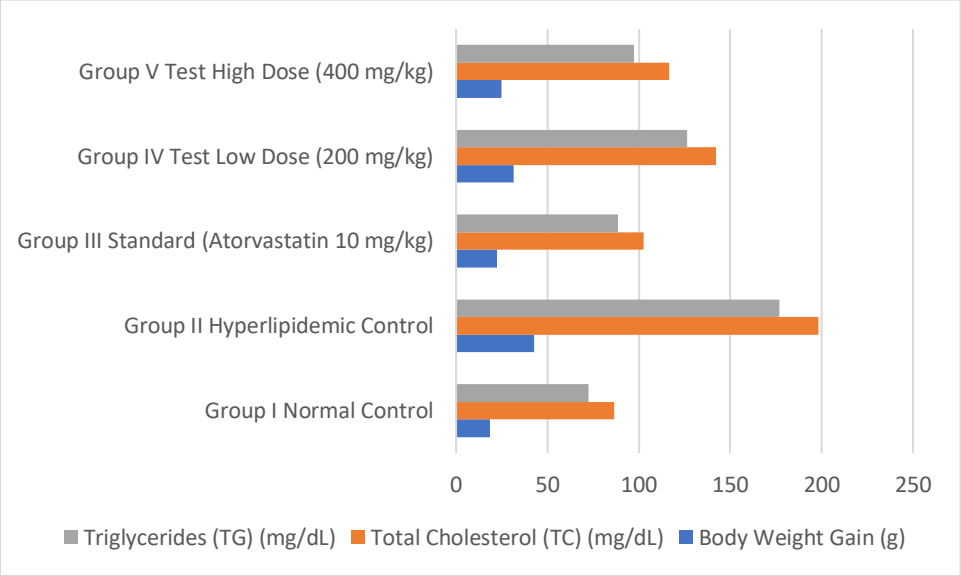
$$Percentage\ of\ yield = \frac{26g}{200g} \times 100 = 13\%$$

50gms of drug dissolved in 250ml of solvent in 1:4 Ratio

Effect of *Terminalia arjuna* Extract on Body Weight, Total Cholesterol, and Triglycerides in HFD-Induced Hyperlipidemic Rats

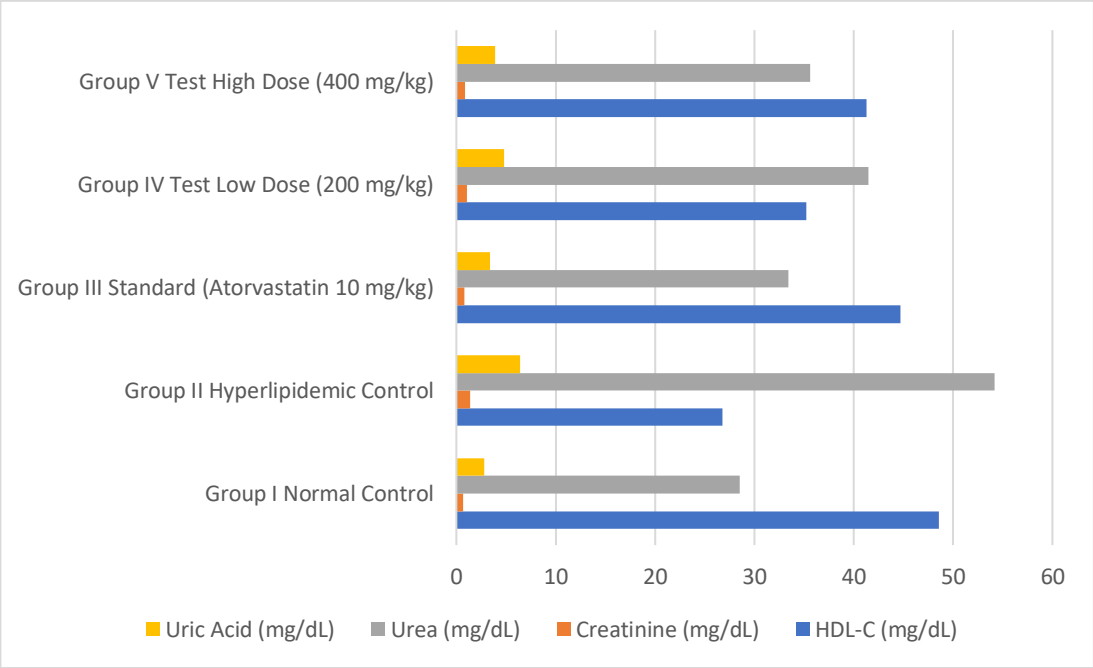
Groups	Body Weight Gain (g)	Total Cholesterol (TC) (mg/dL)	Triglycerides (TG) (mg/dL)
Group I Normal Control	18.4 ± 1.2	86.5 ± 3.1	72.4 ± 2.8
Group II Hyperlipidemic Control	42.8 ± 2.4	198.4 ± 5.6	176.9 ± 5.2
Group III Standard (Atorvastatin 10 mg/kg)	22.3 ± 1.5	102.7 ± 3.8	88.5 ± 3.2
Group IV Test Low Dose (200 mg/kg)	31.6 ± 1.9	142.3 ± 4.5	126.4 ± 4.1
Group V Test High Dose (400 mg/kg)	24.8 ± 1.6	116.8 ± 3.9	97.2 ± 3.5

The hyperlipidemic control group showed a significant increase in body weight, total cholesterol, and triglyceride levels compared with the normal control group due to administration of a high-fat diet. Treatment with atorvastatin and Terminalia arjuna ethanolic extract significantly reduced body weight gain and serum lipid levels. The high-dose extract (400 mg/kg) produced greater reduction in cholesterol and triglyceride levels than the low-dose extract, indicating dose-dependent antihyperlipidemic activity.



Groups	HDL-C (mg/dL)	Creatinine (mg/dL)	Urea (mg/dL)	Uric Acid (mg/dL)
Group I Normal Control	48.6 ± 2.1	0.68 ± 0.04	28.5 ± 1.6	2.8 ± 0.2
Group II Hyperlipidemic Control	26.8 ± 1.4	1.42 ± 0.08	54.2 ± 2.7	6.4 ± 0.3
Group III Standard (Atorvastatin 10 mg/kg)	44.7 ± 2.0	0.81 ± 0.05	33.4 ± 1.8	3.4 ± 0.2
Group IV Test Low Dose (200 mg/kg)	35.2 ± 1.8	1.06 ± 0.06	41.5 ± 2.0	4.8 ± 0.3
Group V Test High Dose (400 mg/kg)	41.3 ± 1.9	0.88 ± 0.04	35.6 ± 1.7	3.9 ± 0.2

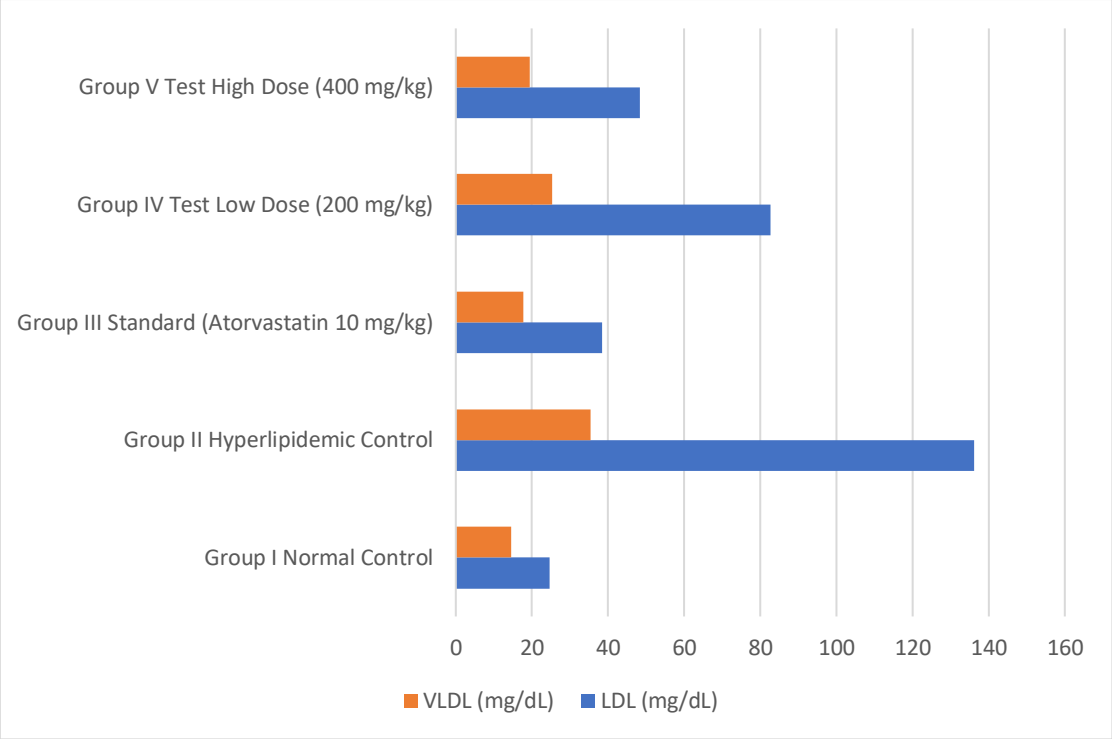
The hyperlipidemic control group showed a marked decrease in HDL-C levels along with significant elevation in creatinine, urea, and uric acid levels compared with the normal control group, indicating altered lipid metabolism and renal dysfunction induced by the high-fat diet. Treatment with atorvastatin and Terminalia arjuna ethanolic extract significantly improved HDL-C levels and reduced elevated renal biochemical parameters. The high-dose extract (400 mg/kg) demonstrated better protective activity than the low-dose extract and showed results comparable to the standard drug.



Effect of *Terminalia arjuna* Extract on LDL and VLDL Levels in HFD-Induced Hyperlipidemic Rats.

Groups	LDL (mg/dL)	VLDL (mg/dL)
Group I Normal Control	24.6 ± 1.5	14.5 ± 0.6
Group II Hyperlipidemic Control	136.2 ± 4.3	35.4 ± 1.0
Group III Standard (Atorvastatin 10 mg/kg)	38.5 ± 1.9	17.7 ± 0.7
Group IV Test Low Dose (200 mg/kg)	82.6 ± 3.1	25.3 ± 0.8
Group V Test High Dose (400 mg/kg)	48.4 ± 2.2	19.4 ± 0.7

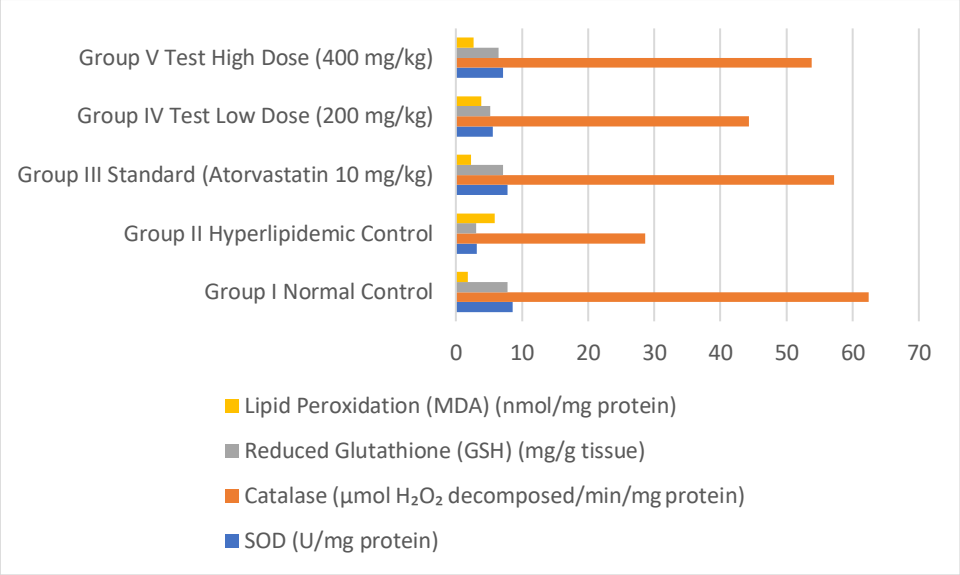
The hyperlipidemic control group exhibited a significant increase in LDL and VLDL levels compared with the normal control group due to high-fat diet administration. Treatment with atorvastatin and Terminalia arjuna ethanolic extract significantly reduced LDL and VLDL levels. The high-dose extract (400 mg/kg) showed greater antihyperlipidemic activity than the low-dose extract and produced results comparable to the standard drug, indicating effective control of abnormal lipid metabolism.



Effect of *Terminalia arjuna* Extract on Antioxidant Parameters in HFD-Induced Hyperlipidemic Rats

Parameters	Group I Normal Control	Group II Hyperlipidemic Control	Group III Standard (Atorvastatin 10 mg/kg)	Group IV Test Low Dose (200 mg/kg)	Group V Test High Dose (400 mg/kg)
SOD (U/mg protein)	8.6 ± 0.4	3.2 ± 0.2	7.8 ± 0.3	5.6 ± 0.3	7.1 ± 0.3
Catalase (µmol H_2O_2 decomposed/min/mg protein)	62.4 ± 2.5	28.6 ± 1.4	57.2 ± 2.1	44.3 ± 1.8	53.8 ± 2.0
Reduced Glutathione (GSH) (mg/g tissue)	7.8 ± 0.3	3.1 ± 0.2	7.1 ± 0.3	5.2 ± 0.2	6.5 ± 0.3
Lipid Peroxidation (MDA) (nmol/mg protein)	1.8 ± 0.1	5.9 ± 0.3	2.3 ± 0.1	3.8 ± 0.2	2.7 ± 0.1

The hyperlipidemic control group showed a significant decrease in antioxidant enzyme levels such as SOD, catalase, and GSH with a marked increase in lipid peroxidation (MDA) compared with the normal control group, indicating oxidative stress induced by the high-fat diet. Treatment with Terminalia arjuna ethanolic extract significantly improved antioxidant enzyme levels and reduced lipid peroxidation. The high-dose extract (400 mg/kg) exhibited stronger antioxidant activity than the low-dose extract and showed results comparable to the standard drug treatment.



DISCUSSION:

The present investigation was undertaken to evaluate the antihyperlipidemic and antioxidant potential of Terminalia arjuna ethanolic extract in high-fat diet (HFD)-induced hyperlipidemic rats. Hyperlipidemia is one of the major risk factors associated with cardiovascular disorders, obesity, fatty liver disease, and oxidative stress. In the present study, administration of HFD for the experimental period successfully induced hyperlipidemia, as evidenced by significant increases in body weight, serum total cholesterol (TC), triglycerides (TG), LDL, VLDL, liver enzyme markers, and renal biochemical parameters, along with a reduction in HDL cholesterol levels in the hyperlipidemic control group.

The increase in body weight observed in HFD-fed animals may be due to excessive accumulation of lipids and altered fat metabolism. Elevated serum cholesterol and triglyceride levels indicate impaired lipid metabolism and increased synthesis or decreased clearance of lipoproteins. LDL and VLDL levels were markedly elevated in hyperlipidemic animals, whereas HDL levels were significantly reduced. Increased LDL and VLDL concentrations are associated with the deposition of cholesterol in blood vessels and the development of atherosclerosis, while HDL plays a protective role by transporting excess cholesterol from peripheral tissues to the liver for excretion.

Treatment with *Terminalia arjuna* ethanolic extract significantly improved the altered lipid profile in experimental animals. Both low-dose and high-dose extract treatment reduced TC, TG, LDL, and VLDL levels and increased HDL levels when compared with the hyperlipidemic control group. The high-dose extract (400 mg/kg) showed greater activity and produced effects comparable to the standard drug atorvastatin. The antihyperlipidemic activity of the extract may be attributed to the presence of bioactive phytoconstituents such as flavonoids, tannins, phenolic compounds, saponins, and triterpenoids, which are known to possess lipid-lowering and antioxidant properties.

Liver function markers such as AST, ALT, and ALP were significantly elevated in the hyperlipidemic control group, indicating hepatic damage caused by excessive lipid accumulation and oxidative stress. Treatment with *Terminalia arjuna* extract significantly reduced these elevated enzyme levels, suggesting hepatoprotective activity. Similarly, renal function parameters including creatinine, urea, and uric acid were elevated in HFD-induced rats, indicating impaired kidney function. Administration of the extract restored these parameters toward normal values, demonstrating nephroprotective effects.

Oxidative stress is considered an important factor in the pathogenesis of hyperlipidemia and associated complications. In the present study, antioxidant enzymes such as superoxide dismutase (SOD), catalase, and reduced glutathione (GSH) were significantly decreased in hyperlipidemic animals, whereas lipid peroxidation levels (MDA) were increased. These findings indicate increased free radical generation and oxidative damage in HFD-fed rats. Treatment with *Terminalia arjuna* extract significantly enhanced antioxidant enzyme levels and reduced lipid peroxidation, indicating potent antioxidant activity. The antioxidant effect may be due to the free radical scavenging and membrane-stabilizing properties of phenolic and flavonoid compounds present in the plant extract.

The study support the traditional use of *Terminalia arjuna* in the management of cardiovascular and metabolic disorders. The extract demonstrated significant antihyperlipidemic, antioxidant,

hepatoprotective, and nephroprotective activities in HFD-induced hyperlipidemic rats. Among the tested doses, the high-dose extract exhibited superior pharmacological activity, suggesting a dose-dependent therapeutic effect. Therefore, *Terminalia arjuna* may serve as a potential natural therapeutic agent for the management of hyperlipidemia and its associated complications.

CONCLUSION:

The present study demonstrated that the ethanolic extract of Terminalia arjuna possesses significant antihyperlipidemic and antioxidant activity in high-fat diet-induced hyperlipidemic rats. Administration of the high-fat diet resulted in marked increases in body weight, serum lipid profile parameters, liver enzymes, renal biochemical markers, and oxidative stress, along with a reduction in HDL cholesterol and antioxidant enzyme levels. Treatment with *Terminalia arjuna* extract effectively restored these altered biochemical parameters toward normal values.

The extract significantly reduced total cholesterol, triglycerides, LDL, VLDL, creatinine, urea, uric acid, AST, ALT, and ALP levels while increasing HDL cholesterol, SOD, catalase, and GSH levels. The reduction in lipid peroxidation further confirmed its antioxidant potential. Among the tested doses, the high-dose extract (400 mg/kg) exhibited greater pharmacological activity and produced effects comparable to the standard drug atorvastatin.

The observed therapeutic effects may be attributed to the presence of phytoconstituents such as flavonoids, tannins, phenolic compounds, saponins, and triterpenoids present in the extract. Overall, the findings suggest that *Terminalia arjuna* possesses potent antihyperlipidemic, antioxidant, hepatoprotective, and nephroprotective properties and may serve as a promising natural agent for the management of hyperlipidemia and related metabolic disorders.

REFERENCES:

1. Singh RB, Niaz MA, Ghosh S. Hypolipidemic and antioxidant effects of Commiphora mukul as an adjunct to dietary therapy in patients with hypercholesterolemia. Cardiovasc Drugs Ther. 1994;8(4):659-664.
2. Thompson-Coon J, Ernst E. Herbs for serum cholesterol reduction: a systematic review. J Fam Pract. 2003;52(6):468-478.
3. Silagy C, Neil A. A meta-analysis of the effect of garlic on serum lipids. J Fam Pract. 1994;38(2):158-162.
4. Gerards MC, Terlou RJ, Yu H, Koks CH, Gerdes VE. Red yeast rice for hyperlipidemia: a meta-analysis. Atherosclerosis. 2015;240(2):415-423.
5. Heshmat-Ghahdarijani K, Mashayekhiasl N, Amerizadeh A, et al. Effect of fenugreek consumption on serum lipid profile: a systematic review and meta-analysis. Phytother Res. 2020;34(9):2230-2245.
6. Ansary J, Forbes-Hernández TY, Gil E, Cianciosi D, Zhang J, Elexpuru-Zabaleta M, et al. Potential health benefit of garlic. Nutrients. 2020;12(6):1-28.
7. Sharma MS, Choudhary PR. Effect of fenugreek seeds on hyperlipidemia in rabbits. J Diet Suppl. 2017;14(1):1-8.
8. Takkella N, Konuri A, Nayak CD, et al. Fenugreek seed extract in high-fat diet rats. J Taibah Univ Med Sci. 2019;14(4):383-389.
9. Nematollahi S, Pishdad GR, Zakerkish M, et al. The effect of berberine and fenugreek seed co-supplementation on lipid profile in type 2 diabetes mellitus: a randomized clinical trial. Diabetol Metab Syndr. 2022;14:120.
10. Studies documenting Allium sativum (garlic) for lipid lowering — pooled RCT data (multiple sources summarized in reviews above).
11. Classic experiments on Commiphora mukul lipid effects — see Satyavati GV et al., Indian J Med Res. 1969;50:1950-1955.
12. Gylling H, Plat J, Turley S, et al. Plant sterols and stanols in the management of dyslipidemia and prevention of cardiovascular disease. Atherosclerosis. 2014;232:346-360.
13. Abumweis SS, Barake R, Jones PJH. Plant sterols/stanols as cholesterol lowering agents: a meta-analysis of randomized controlled trials. J Clin Lipidol. 2008;2(2):199-208.
14. Katan MB, Grundy SM, Jones P, Law M, Miettinen T, Paoletti R. Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. Mayo Clin Proc. 2003;78(8):965-978.
15. Plat J, Mensink RP. Plant stanol and sterol esters in the control of blood cholesterol levels: mechanism and safety aspects. Am J Cardiol. 2002;90(10C):50G-58G.
16. Visavadiya NP, Narasimhacharya AVRL. Hypolipidemic and antioxidant effects of functional foods. J Sci Food Agric. 2008;88(11):1879-1883.
17. Park J, et al. Hypolipidemic effect of Cynara scolymus (artichoke) extract in animals. J Med Food. 2009;12(3):558-564.
18. Gardner CD, et al. Effect of various garlic preparations on lipid profiles: systematic evaluation. J Nutr. 2007;137(3 Suppl):792S-799S.
19. Rouhi-Boroujeni H, et al. Medicinal herbs with hypolipidemic effects: mechanisms and clinical evidence. Phytother Res. 2017;31(11):1671-1687.

20. Mehraban S, et al. Systematic review of meta-analyses on herbal medicines for dyslipidemia. Phytother Res. 2021;35(10):5803-5817.
21. Sedighi M, et al. Phytochemicals with potential in lipid control and atherosclerosis prevention. J Ethnopharmacol. 2017;198:373-397.
22. Parikh P, et al. Indigenous medicinal plants used to treat metabolic syndrome components including dyslipidemia: a review. J Ethnopharmacol. 2014;155(2):1010-1020.
23. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical Chemistry*. 1982;28(10):2077–2080.
24. Burstein M, Scholnick HR, Morfin R. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. Journal of Lipid Research. 1970;11(6):583–595.
25. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clinical Chemistry*. 1972;18(6):499–502.
26. Jaffe M. Über den Niederschlag welchen Pikrinsäure in normalem Harn erzeugt und über eine neue Reaction des Kreatinins. Zeitschrift für Physiologische Chemie. 1886;10:391–400.
27. Fawcett JK, Scott JE. A rapid and precise method for the determination of urea. *Journal of Clinical Pathology*. 1960;13:156–159.
28. Caraway WT. Determination of uric acid in serum by a carbonate method. *American Journal of Clinical Pathology*. 1955;25(7):840–845.
29. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*. 1957;28(1):56–63.
30. Kind PRN, King EJ. Estimation of plasma phosphatase by determination of hydrolyzed phenol with amino-antipyrine. Journal of Clinical Pathology. 1954;7(4):322–326.
31. Misra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*. 1972;247(10):3170–3175.