TO EVALUATE THE ANTI-ATHEROSCLEROTIC POTENTIAL OF QUERCETIN IN ALLOXAN-INDUCED DIABETIC RATS FED WITH HIGH-FAT DIET

BIPLAV S, SINDHURA G, SHIVALINGE GOWDA K P*

Department of Pharmacology, PES College of Pharmacy, Hanumanthanagar, Bengaluru – 560 050, Karnataka, India.

Email: shivalinge65@gmail.com

ABSTRACT

Objective: The main aim of the present study is concerned with the evaluation of anti-atherosclerotic potential of quercetin in alloxan-induced diabetic rats fed with high-fat diet (HFD).

Methods: Atherosclerosis (AS) is the major cause for many of the cardiovascular disease, and it is accelerated in the presence of diabetes mellitus and causes profound alterations in the lipid profile. The method used for the induction of AS was using HFD for 60 days. In this study, rats were divided into four groups (n=6). Group I served as normal control, Group II alloxan (120 mg/kg b.w i.p)-treated diabetic rats, Group III received quercetin (50 mg/ kg b.w p.o), and Group IV received atorvastatin (10 mg/kg b.w p.o) along with alloxan (120 mg/kg b.w i.p) on the 1st day of the days of the study period. AS was induced in Group II, Group III, and Group IV rats by feeding them with HFD from the 1st day to 60th day. The body weight, feed intake was measured daily. The blood was withdrawn from retro-orbital plexus, and the serum was used for the estimation of lipid profile (total cholesterol [TC], triglycerides [TGs], low-density lipoprotein cholesterol [LDL-C], very LDL-C [VLDL-C], and high-density lipoprotein cholesterol [HDL-C]). After scarification under overdose of ketamine, the histopathological study of aorta was carried out.

Results: The results showed that the quercetin-treated rats showed a decrease in body weight gain, decreased levels of TC, TGs, LDL-C, and VLDL-C, and increased levels of HDL-C were observed in Group III rats when compared to alloxan-induced diabetic rats fed with HFD (Group III). The histopathological study of aorta showed no development of plaques and of foam cells.

Conclusion: From this study, it can be calculated that quercetin has anti-atherosclerotic activity as it significantly altered overall lipid profile in diabetic rats fed with HFD. This activity may be attributed to its antioxidant, inhibition of HMG-CoA reductase activity of quercetin.

Keywords: Alloxan, Quercetin, High-fat diet, Serum lipid profile, Atorvastatin, Atherosclerosis, Diabetes.

INTRODUCTION

Atherosclerosis (AS) is a disease caused due to inflammatory response and mainly due to the deposition of lipids mainly the cholesterol and its esters, blood components, and carbohydrates in the intima of the artery and its branches accompanied with the deposition of calcium and connective tissue in the surrounding tissue. AS is characterized by the movement and migration of medial smooth muscle cells in the intima of the artery and proliferation of such cells causes thickening of intima, hardening, and consequently, the appearance and formation of atherosclerotic plaques and fibrous fatty deposits [1].

One of the primary risk factors for AS is the concentration of cholesterol particularly the low-density lipoprotein cholesterol (LDL-C) on the higher side. Other risk factors include smoking, diabetes mellitus (DM), hypertension, and sedentary lifestyle. DM is one of the major contributors characterized by hyperglycemia, altered metabolism of lipids, carbohydrates, and proteins and leads to increased risk of complications from vascular disease [2]. High concentration of cholesterol, particularly the LDL-C is one of the principal risk factors. The atherosclerotic lesions contain large number of immune cells and T-cells. Further, the disease is associated with systemic immune response and signs of inflammation [3]. AS is reported to cause myocardial and cerebral infractions; hence, it is considered to be the main clinical syndromes associated with AS and is the leading cause of death in the world. Diabetes is associated with a marked increase (by a factor of two to four) in the risk of coronary heart disease (CHD). Coronary artery disease (CAD) arised from AS is the leading cause of morbidity and mortality worldwide. According to the recent report,

AS is the leading contributors for morbidity and mortality. The United States estimated of about one-third deaths in a year, also it is reported that 12 million Americans have CAD, 4 million stroke patients [4].

At present, available therapies for AS include atorvastatin, simvastatin, clofibrate, cholesterol, cholestyramine, niacin, niacin, ezetimibe. However, these drugs process a number of limitations such as adverse effects and high rates of secondary failure. The plant kingdom holds great potential to meet this need. Hence, the use of herbal medicine for the treatment of disease has been used by man for many years and has been practiced even today [5]. Quercetin is a bioflavonoid belonging to the major class of flavanol (sub-family of flavonoids) found in abundantly in apple, onions, blueberries, cranberry, sweet potato, strawberries, etc. Quercetin is also an important bioactive constituent of human diet and acts as an antioxidant through free radical scavenging action and by interacting with various endogenous proteins. Anti-atherogenic activity may be attributed to its antioxidant, anti-inflammatory, antitumor, antiviral, and inhibition of phosphodiesterase enzyme activity [6]. The present study was done to study the possible anti-atherosclerotic effects of quercetin in diabetic rats.

METHODS

Animals

Adult male Wistar rats (180–200±20 g) were procured from authenticated supplier, Adita Biosys Private Limited, Tumakuru, Karnataka, India. All the animals were housed under standard laboratory conditions, maintained on a 12 h light:12 h dark cycle, and food and water were provided *ad libitum*. Animals were acclimatized for 7 days to

laboratory conditions before the test. The experimental protocols were approved by IAEC (Ref No:-PESCP/IAEC/25/2015. Dated:-29/12/2015) and conducted according to CPCSEA guidelines (CPCSEA Reg No:-600/PO/Ere/S/02CPCSEA), Government of India. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Drugs and chemicals

Alloxan hydrate, purified cholesterol, and quercetin dehydrate were purchased from High Purity Laboratory Chemicals Pvt. Ltd, Mumbai. Atorvastatin (Stator 10) manufactured by Abbott Healthcare Pvt., Ltd., which was used as standard drug was purchased from a local pharmacy store. All the other chemicals used in the study were of analytical grade and of highest purity supplied by Merk Life Sciences Pvt., Ltd., Bengaluru, Karnataka, India.
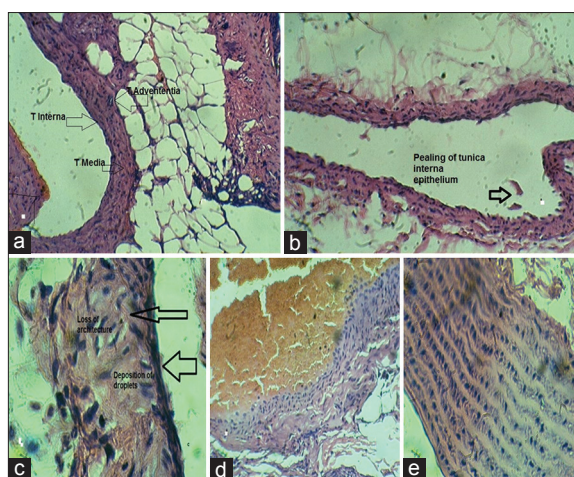
Induction of DM in rats

After 18 h of fasting in rats, stable DM was induced to the Group II, III, and IV rats by single intraperitoneal injection of alloxan (120 mg/ kg body weight) dissolved in 0.1 mol/L of sodium citrate buffer solution (pH 4.5), and the Group I rats (control group) received only the citrate buffer. The rats were given with the standard diet (20% glucose solution for 12 h to prevent initial drug-induced hypoglycemic mortality) and water *ad libitum*. After 72 h of alloxan injection, fasted blood glucose levels were estimated by tail tip method using glucometer. Only the rats with the blood glucose level over ≥150 mg/dL were considered to be diabetic rats and were used for the experiment [7].

Induction of AS in rats

Preparation of high-fat diet (HFD)

The normal standard animal pellets were first crushed in mortar and pestle, and then, it was grinded in a mixer grinder to get the fine powder along with the other ingredients containing cholesterol 2%, cholic acid 1%, sucrose 40%, and coconut oil 10% were added in the ascending order of their quantity and mixed well. The above powdered feed was then mixed with sufficient quantity of water to make the feed shape as small balls which were then stored in refrigerator at 22°C±8°C in a self- seal plastic covers [8].

Fig. 1: Histopathological evaluation of aorta from the groups of: (a) normal, (b and c) control, (d) Quercetin treated, (e) Atorvastatin treated

Pharmacological activities

Animals were divided into four groups containing six rats in each group. Group I (normal) received drinking water throughout the experimental study till 60 days. Group II (disease control) rats received alloxan (120 mg/kg b.w i.p), Group III received quercetin (50 mg/kg b.w p.o) with alloxan (120 mg/kg b.w i.p), and Group IV received atorvastatin (10 mg/kg b.w i.p) with alloxan (120 mg/kg b.w i.p) on the 0th day of the 60 days of the study period. AS was induced in Group II, Group III, and Group IV rats by feeding them with HFD from the 1st day to the 60th day. The treatment with quercetin (test substance) and atorvastatin (standard) was started from the 31st day to the 60th day. Fasting blood samples were collected from the tail vein for blood sugar estimation on the 0th day using one touch glucometer. The food and water intake of each rat was monitored daily and the difference in body weight of individual animals was measured [9].

Biochemical analysis

24 h after the last dosing schedule, the animals were anesthetized using light ketamine anesthesia at the dose of 40 mg/kg b.w i.p. Then, the blood was withdrawn using a capillary tube from retro-orbital plexus. 1 ml of blood was collected from each animal of all the groups in Eppendorf tubes. The collected blood was kept in an upright position for 30–45 min for incubation to facilitate clotting and then subjected to cold centrifugation at 2000 rpm for 20 min. The supernatant serum was collected in another Eppendorf tube and is checked for turbidity. If the turbidity is present, then again it is subjected for recentrifugation at 2000 rpm for 10 min. The separated serum was used for the assay of the biochemical parameters such as total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TGs) were estimated using the diagnostic kits. Low-density lipoprotein cholesterol (LDL-C) and very LDL (VLDL) were calculated using Friedewald's formula. After the biochemical estimations, the animals of all the groups were euthanized by an overdose of ketamine anesthesia [10].

Histopathological investigations

After the animals were euthanized, their aorta was dissected out. Ice was used during the procedure to keep the aorta samples fresh and prevent them from any degradation. The dissected samples of the aorta from each group of animals were collected in 10% v/v formalin solution and were sent to the local pharmacy laboratory for histopathological studies.

Statistical analysis

Values reported were expressed as mean±standard deviation. The statistical analysis was performed using one-way analysis of variance, followed by Bonferroni method of statistics for comparison of selected pairs to compare the treatment groups with the control groups. Calculations were conducted using GraphPad Prism statistical program (version: 5.03). With all analyses, an associated $p < 0.0001$ was considered significant.

RESULTS

Body weight

The diabetic rats fed with HFD (Group II) have shown a significant increase in body weight again (238.3±21.37) as compared with that of normal control (Group I) rats (198±14.72). The Group III rats treated with quercetin showed a significant decrease in body weight gain (200±17.89) when compared with Group II (disease control rats). The Group IV rats treated with atorvastatin also showed a significant

Table 1: Effect of Quercetin (Que) on body weight (b.w.)

Group n=6	Treatment	Initial wt in g	Final wt in g	Change in b.w (%)
I	Normal	147.5±10.37	198±14.72	50.8±9.7
II	Disease control	160±14.29	238.3±21.37	77.5±11.73
III	Que treated	158±11.69	200±17.89	41.67±9.832
IV	Ator treated	160±13.04	195±10.49	36.67±9.832

The values were expressed as mean±SD (n=6) animals in each group. SD: Standard deviation, HFD: High-fat diet

Table 2: Effect of Quercetin on TC

Group	Treatment	TC (mg/dL)
I	Normal	68.37±10.16
II	Disease Control	104.6±6.311***[a]
III	Que treated	66.68±6.129***[b]
IV	Ator treated	58.15±8.414***[b]

The values were expressed as mean±SD (n=6) animals in each group. ***[a]$p<0.0001$ is considered as significant when the column is compared to normal group and***[b]$p<0.0001$ is considered as significant when the columns are compared to HFD group done by Bonferroni's test comparison of selected pair of columns. TC: Total cholesterol, SD: Standard deviation, HFD: High-fat diet

Table 3: Effect of Quercetin on (TGs)

Group	Treatment	TGs mg/dL
I	Normal	76.70±3.332
II	Disease control	124.8±5.656***[a]
III	Que treated	55.57±5.506***[b]
IV	Ator treated	53.27±6.27***[b]

The values were expressed as mean±SD (n=6) animals in each group.***[a]$p<0.0001$ is considered as significant when the column is compared to normal group and ***[b]$p<0.0001$ is considered as significant when the columns are compared to HFD group done by Bonferroni's test comparison of selected pair of columns. TGs: Triglycerides, SD: Standard deviation, HFD: High-fat diet

Table 4: Effect of Quercetin on HDL-C

Group	Treatment	HDL-C mg/dL
I	Normal	44.52±2.603
II	Disease control	5.530±0.6959***[a]
III	Que treated	29.93±1.237***[b]
IV	Ator treated	26.32±1.853***[b]

The values were expressed as mean±SD (n=6) animals in each group***[a]$p<0.0001$ is considered as significant when the column is compared to normal group and ***[b]$p<0.0001$ is considered as significant when the columns are compared to HFD group done by Bonferroni's test comparison of selected pair of columns. SD: Standard deviation, HFD: High-fat diet, HDL-C: High-density lipoprotein cholesterol

decrease in body weight gain (195±10.49) when compared with Group II (disease control rats) (Table 1).

Effect of Quercetin on lipid profile in HFD-induced atherosclerotic diabetic rats

Estimation of serum TC [11]

Serum cholesterol was estimated using Erba cholesterol kit by colorimetric, endpoint (Cholesterol oxidase, peroxidase-4-aminoantipyrene) method. On induction on with HFD, there was significant increase in groups receiving HFD when compared to the group fed with normal diet ($p<0.0001$). The values of serum TC level for HFD groups and normal group were found to be 104.6±6.311 and 68.37±10.16, respectively. The Group III rats treated with quercetin (50 mg/kg b.w p.o) showed a significant decrease in the levels of serum TC (66.68±6.129) and also Group IV rats treated with standard drug atorvastatin (10 mg/kg b.w p.o) showed a significant decrease in the serum TC levels when compared to Group II rats (104.6±6.311) (Table 2).

Estimation of serum TGs [12]

Serum TGs were estimated using the Erba TG kit by Enymatic, endpoint, colorimetric, GPO/Trinder (Glycerol-3-phosphate, N-Ethyl-N-sulfopropyl-n-anisidine) method. The Group II rats were fed with HFD showed a significant elevated level of TG's (124.8±5.656) when compared to the group fed with normal chow diet (76.70±3.322). The Group III rats treated with quercetin showed a significant decrease in the serum TGs levels (55.57±5.656) and also the Group IV rats treated

with the standard drug atorvastatin showed a significant reduction in the levels of TGs when compared with the Group II rats (124.8±5.656) (Table 3).

Estimation of serum HDL-C [13]

Serum HDL was estimated using Erba cholesterol kit by the phosphotungstic acid method. The Group II rats fed with HFD showed a significant decrease in the levels of HDL-C (5.530±0.6959) when compared to the group fed with normal chow diet (44.52±2.630). The Group III rats treated with quercetin showed a significant increase in the serum HDL-C (29.93±1.237) when compared to the Group II rats (5.530±0.6959) and also the Group IV rats treated with the standard drug atorvastatin showed a significant increase in HDL-C levels when compared with the Group II rats (5.530±0.6959) (Table 4).

Estimation of serum LDL-C [14]

Serum LDL-C was estimated using Friedewald's and Fredrickson's formula. The Group II rats fed with HFD showed a significant elevation in the levels of LDL-C (24.27±1.556) when compared to the group fed with normal chow diet (15.42±0.7707). The Group III rats treated with quercetin showed a significant reduction in the serum LDL-C (13.51±3.393) when compared to the Group II rats (24.27±1.556) and also the Group IV rats treated with the standard drug atorvastatin showed a significant reduction in HDL-C (23.74±5.635) levels when compared with the Group II rats (24.27±1.556) (Table 5).

Estimation of serum VLDL-C [15]

VLDL cholesterol was calculated using the following formula.

$$VLDL = \frac{Triglyceride}{5}$$

The Group II rats fed with HFD showed a significant elevation in the levels of VLDL-C (24.27±1.556) when compared to the group fed with normal chow diet (15.42±0.7707). The Group III rats treated with quercetin showed a significant reduction in the serum VLDL-C (13.45±1.621) when compared to the Group II rats (24.27±1.556) and also the Group IV rats treated with the standard drug atorvastatin showed a significant reduction in HDL-C (13.51±3.393) levels when compared with the Group II rats (24.27±1.556) (Table 6).

Effect on atherogenic index (AI)

The AI was calculated using the formula.

$$AI = \frac{TC - HDL(C)}{HDL(C)}$$

The Group II rats administered with HFD showed significant change elevated level of AI (13.14±3.158) when compared with normal rats fed (0.6817±0.02787) with normal chaw pellet. Group III rats (3.849±0.7593) treated with quercetin and Group IV rats (1.597±0.7068) treated with standard drug atorvastatin showed significant reduction in AI when compared with Group II rats (Table 7).

Histopathological study of aorta

The histopathological examination of aorta is intended to study the blood vessel condition in relation to the occurrence of AS in experimental animals. The AS is descriptively observed by identifying the existence of foam cells in the rat's aorta. Foam cells are formed by the presence of fatty deposit in the macrophages around the cell's tunica intima. In normal rats, the epithelial layers tunica interna, tunica media, and tunica adventitia of aortic wall are arranged in systematic manner providing proper integrity to blood vessel (a). In disease control group, the aortic wall reveals loss of epithelial integrity in internal layers (b). The aortic wall also reveals loss of epithelial integrity in internal layers, degenerative changes in the medial layers, and fat like deposits observed on the inner wall of the blood vessel (c). The histopathological conditions of Group III rats treated with quercetin (50 mg/kg b.w.) have shown to reduce the plaque area around the aorta (d). The

Table 5: Effect of Quercetin on LDL-C

Group	Treatment	LDL-C mg/dL
I	Normal	24.14±6.951
II	Disease control	66.58±16.97***[a]
III	Que treated	29.28±13.50***[b]
IV	Ator treated	23.74±5.635***[b]

The values were expressed as mean±SD (n=6) animals in each group.***[a]p<0.0001 is considered as significant when the column is compared to normal group and ***[b]p<0.0001 is considered as significant when the columns are compared to HFD group done by Bonferroni's test comparison of selected pair of columns. SD: Standard deviation, HFD: High-fat diet, LDL-C: Low-density lipoprotein cholesterol

Table 6: Effect of Quercetin on VLDL-C

Group	Treatment	VLDL-C mg/dL
I	Normal	15.42±0.7707
II	Disease control	24.27±1.556***[a]
III	Que treated	13.45±1.621***[b]
IV	Ator treated	13.51±3.393***[b]

The values were expressed as mean±SD (n=6) animals in each group.***[a]p<0.0001 is considered as significant when the column is compared to normal group and ***[b]p<0.0001 is considered as significant when the columns are compared to HFD group done by Bonferroni's test comparison of selected pair of columns. SD: Standard deviation, HFD: High-fat diet, VLDL-C: Very low-density lipoprotein cholesterol

Table 7: Effect of Quercetin on AI

Group	Treatment	AI
I	Normal	0.6817±0.02787
II	Disease control	13.14±3.158***[a]
III	Que treated	3.849±0.7593***[b]
IV	Ator treated	1.597±0.7068***[b]

The values were expressed as mean±SD (n=6) animals in each group.***[a]p<0.0001 is considered as significant when the column is compared to normal group and ***[b]p<0.0001 is considered as significant when the columns are compared to HFD group done by Bonferroni's test comparison of selected pair of columns. SD: Standard deviation, HFD: High-fat diet, AI: Atherogenic index

histopathological conditions of rats treated with atorvastatin (10 mg/kg bw) reveal layers of aorta appeared apparently normal with distinct layers without plaques and foam cells, but there was poorer regeneration of the damaged tissue (e) (Fig. 1).

DISCUSSION

It is very well documented that hyperlipidemia, and thereby AS is a well-known complication of DM and it is characterized by the coexistence of hyperglycemia and abnormal increase in the lipid profile of cholesterol, TGs, phospholipids, and lipoproteins. It is a major area of concern of study of lipid profile in diabetes as it is widely acknowledged from study reports about the rate of incidence of atherosclerotic, and vascular disease is a major cause of premature mortality in patients with diabetes.

The prevention and control of hyperlipidemia is a prerequisite need for the prevention of diabetic complications such as diabetic retinopathy, diabetic nephropathy, diabetic neuropathy, ischemic heart disease, cerebrovascular disease, and arteriosclerosis obliterans in diabetes. Studies have also revealed that treatment with insulin for the normalization of plasma glucose levels did not restore the normalization of HDL-C in fact risk factors along with hyperglycemia are causing lowering of HDL-C.

It has also been reported that glycated HDL clearance is accelerated in circulation in contrast to the effect of glycated LDL whose catabolic rate is reduced. The accelerated clearance of HDL was observed even with mild glycation which was suggested as a contributing cause of low plasma levels of HDL in diabetic patients and hence works as an underlying factor for increased risk of AS in diabetic patients [16].

In this present study, the Group II rats were fed with HFD showed significant increase in body weight as compared to Group I rats, whereas Group III and Group IV rats treated with quercetin and atorvastatin, respectively, show reduction of body weight gain as compared with Group II rats, the underlying mechanism involves in the weight reduction of Group III rats treated with quercetin may be attributed to its antihyperlipidemic/antiobesity effects of quercetin involve in hepatic regulation of lipid metabolism [17]. Hypercholesterolemia, hypertriglyceridemia, hyperlipidemia, and resulting AS have been implicated in the pathophysiology of CHD and myocardial infarction. The reduction in TGs, TC, and lipoproteins such as LDL, VLDL may decrease the risk of CVD, and therefore, enormous efforts have been extended to achieve this aim. In this present study, the Group II rats showed increased levels of TC and TGs as compared to Group I rats. Group III rats treated with quercetin significantly reduce the serum TC and TG when compared with HFD-induced Group II rats. Flavonoids like quercetin are powerful antioxidant resulting in the inhibition of LDL oxidation also polyphenols like quercetin causes alteration in hepatic cholesterol absorption, TG assembly and secretion through inhibition of phosphodiesterase in the adipose tissue and liver. Elevated levels of LDL-C and VLDL-C are often accompanied by premature AS and CVD. The low level of HDL-C is an important risk factor for CVD. The role of HDL-C as a cardioprotective has been attributed to its role in reverse cholesterol transport, its effect on endothelial cells and as antioxidant [18]. In general, flavonoids like quercetin can increase HDL-C and also decreases LDL-C. HFD increases serum LDL levels and oxidative stress and thereby increases atherosclerotic plaque formation. From the present study, it is evident like HFD-induced Group II rats showed increased LDL and VLDL levels with concomitant reduction in serum HDL-C level when compared with Group I rats. Group III rats treated with quercetin showed reduced levels of LDL and VLDL and increased serum HDL-C level when compared with Group II rats which could be due to reduction of serum TC and increase LDL receptor activity by quercetin, and it can be presumed that the reduction of TC by quercetin could have been associated with a reduction of its LDL fraction which is the target for several hypolipidemic drugs. LDL/ HDL and TC/ HDL ratio have direct correlation with CVD. An increase in LDL/ HDL and TC/HDL ratio is directly proportional to the increased risk of CVD. From our study, we observe that HFD fed Group II rats showed increased AI, LDL/HDL, and TC/HDL when compared with Group I rats. Group III rats treated with quercetin decreases LDL/ HDL, TC/HDL, and AI due to its action on reducing HMG-CoA reductase activity an enzyme required for cholesterol synthesis [19].

CONCLUSION

The findings of the current study suggest that quercetin displays anti-atherosclerotic potential (antihypercholesterolemic, anti-hypertriglyceridemic, and antihyperlipidemic) effect by significant reduction of serum TC, serum TG, serum LDL-C, serum VLDL-C, and increased HDL-C. Quercetin might have mediated their activity, i.e.,anti-atherosclerotic potential by interfering with lipid biosynthesis, i.e.,inhibiting HMG-CoA reductase activity, antioxidant potential, and alteration in TG secretion and assembly as well as inhibition phosphodiesterase in adipose tissue and liver.

However, the precise mechanism of action is yet to be established, and further, research is warranted toward studying the fractions and isolated chemical compounds for anti-AS like activities.

REFERENCES

1. Joshi SC, Jain PK. A review on hypolipidaemic and antioxidant potential of some medicinal plants. World J Pharm Pharm Sci 2014;3:357-80.
2. Suman M, Shivalinge GK, Paul U, Priyanka S. Evaluation of antidiabetic and antihyperlipidemic activity of newly formulated polyherbal antidiabetic tablets in streptozocin-induced diabetes mellitus in rats.

Asian J Pharm Clin Res 2016;9:202-7.

3. Martha S, Akula A, Reddy N. Antiatherosclerotic model of atorvastatin and clopidogrel alone and in combination in rats. Indian J Exp Biol 2008;46:698-703.

4. Pamidiboina V, Razdan R, Hariprasad MG. Evaluation of the antihyperlipidemic, cardioprotective activity of a polyherbal formulation. Int J Pharm Pharm Sci2010;2:1-3.

5. Neal RC, Jones PH. Complementary therapy to target LDL cholesterol: The role of the ezetimibe/simvastatin combination. Vasc Health Risk Manag 2006;2:31-8.

6. Seiva FR, Chuffa LG, Braga CP, Amorim JP, Fernandes AA. Quercetin ameliorates glucose and lipid metabolism and improves antioxidant status in postnatally monosodium glutamate-induced metabolic alterations. Food Chem Toxicol 2012;50:3556-61.

7. Rajaram K. Antioxidant and antidiabetic activity of tectonagrandislinn. In alloxan induced albino rats. Asian J Pharm Clin Res 2013;6:174-7.

8. Rajasekaran S, Anandan R, Nishad KM. Anti hyperlipidemic activity of acalyphaindicalinn. On atherogenic diet induced hyperlipidemia. Int J Pharm Pharm Sci 2013;5:699-701.

9. El-Nekeety AA, Abdel-Azeim SH, Hassan AM, Hassan NS, Aly SE, Abdel-Wahhab MA, *et al.* Quercetin inhibits the cytotoxicity and oxidative stress in liver of rats fed aflatoxin-contaminated diet. Toxicol Rep 2014;1:319-29.

10. Sarvesh CN, Fernandes J. Evaluation of antihyperlipidemic activity of leaves of *Achyranthesaspera* linn. Using hyperlipidemic rats. Asian J Pharm Clin Res 2017;10:211-5.

11. Searcy RL. Diagnostic Biochemistry. New York, NY: McGraw-Hill; 1969.

12. Cole, TG, Klotzsch SG, McNamara J. Measurement of triglyceride concentration. In: Rifai N, Warnick GR, Dominiczak MH, editors. Handbook of Lipoprotein Testing. Washington, DC: AACC Press; 1997. p. 155-26.

13. Burtis CA, Ashwood ER, Bruns DE. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 5th ed. Hudson, OH: WB Saunders Comp; 2012.

14. Fukuyama N, Homma K, Wakana N, Kudo K, Suyama A, Ohazama H, *et al.* Validation of the friedewald equation for evaluation of plasma LDL-cholesterol. J Clin Biochem Nutr 2008;43:1-5.

15. Mahmoud B, Hetta M, Etwab SA, Hassan S. Improvement of lipid profile and antioxidant hypercholesterolemic albino rats by polysaccharide extracted from the green alga Lactucalinnaeus. Saudi J Biol Sci 2011;18:333-40.

16. Zakaria Z, Das S, Lin TS, Thent CZ. Histological changes in the heart and the proximal aorta in experimental diabetic rats fed with Piper sarmentsou. Afr J Tradit Complement Altern Med 2012;9:396-40.

17. Andallu B, Vinay Kumar AV, Varadacharyulu NCh. Lipid abnormalities in streptozotocin-diabetes: Amelioration by *Morus indica* L. Cv suguna leaves. Int J Diabetes Dev Ctries 2009;29:123-8.

18. Hoek-van den Hil EF, van Schothorst EM, van der Stelt I, Swarts HJ, Venema D, Sailer M, *et al.* Quercetin decreases high-fat diet induced body weight gain and accumulation of hepatic and circulating lipids in mice. Genes Nutr 2014;9:418.

19. Kamesh V, Sumathi T. Antihypercholesterolemic effect of *Bacopa monniera* linn. On high cholesterol diet induced hypercholesterolemia in rats. Asian Pac J Trop Med 2012;5:949-55.