J. Pharm. Res. 2023;22(1):21–26

Print ISSN: 0973-7200
Online ISSN: 2454-8405

Journal of Pharmaceutical Research

Research Article

A Study of the Antihyperlipidemic and Antioxidant Activities of Ethanolic Extracts of Leaves of *Houttuynia cordata* Thunb . (EELHC) in Albino Rats

Bikram Dutta Tassa[1], Navajit Sahu[2,*], Pradumna Pathak[3]

[1]*Associate Professor, Department of Pharmacology, Assam Medical College, Dibrugarh, Assam, India*
[2]*Assistant Professor, Department of Pharmacology, Assam Medical College, Dibrugarh, Assam, India*
[3]*Assistant Professor, Department of Pharmacology, Fakhruddin Ali Ahmed Medical College, Barpeta, Assam, India*

ARTICLE INFO

Article history:
Received 05.02.2023
Revised 23.02.2023
Accepted 28.02.2023
Published 08.05.2023

* *Corresponding author.*
Navajit Sahu
navasahu@gmail.com

https://doi.org/
10.18579/jopcr/v22.1.23.2

ABSTRACT

The present study was designed to evaluate the antihyperlipidemic and antioxidant activities of leaves of *Houttuynia cordata* Thunb. The ethanolic extract was obtained by percolation method and acute oral toxicity tests were performed according to OECD (Organization for Economic Cooperation and Development) guidelines. Hyperlipidaemia was induced by feeding the rats with a high-fat diet consisting of coconut oil and vanaspati ghee, in a ratio of 2:3 v/v at a dose of 10 ml/Kg body weight. The extract was given at a dose of 500mg/kg body weight. Lipid profile, Malondialdehyde (MDA), Catalase (CAT), and Superoxide Dismutase (SOD) were measured using standard methods. The extract showed a significant decrease in total cholesterol, triglycerides, LDL, and MDA in the blood. On the other hand, HDL, CAT, and SOD increased significantly. The study demonstrates that the ethanolic extract of leaves of Houttuynia *cordata Thunb*., decreases blood lipid levels and lipid peroxidation.

Keywords: Antihyperlipidemic; Antioxidant; Houttuynia cordata Thunb; EELHC

INTRODUCTION

Raised serum lipid profile, particularly of cholesterol along with the generation of reactive oxygen species (ROS), plays a key role in the development of coronary artery disease (CAD) and atherosclerosis.[1] Clinical trials showed conclusively that lowering serum cholesterol reduces morbidity and mortality from coronary artery disease in patients with established coronary artery disease and also reduces new coronary artery disease events and mortality in patients without established coronary artery disease.[2] CAD is a serious medical problem that affects millions of people annually throughout the world. People who are predisposed to a combination of risk factors (dietary habits, genetic susceptibility, etc.) are more prone to develop atherosclerosis and CAD.[3]

The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular disease or cerebrovascular disease.[4] A significant number of patients with hypercholesterolemia do not achieve adequate cholesterol reduction with statins and other lipid-lowering drugs.[5]

Although various drugs like HMG-CoA reductase inhibitors, Nicotinic acid, Fibrate derivatives, and bile acid sequestrants are available to combat the situation, they are not always free from side effects. Myopathy, hyperuricemia, peripheral neuropathy, impotence, gynaecomastia, hepatitis, impotence, skin rashes, etc. are noted.[6] For ages, there has been always a search for an alternative to synthetic drugs with maximal efficacy, and safety at a minimal cost.

Phytosterols and natural antioxidants have also been shown to be effective in reducing lipid profiles and also mitigate peroxidative modification of lipoproteins and atherosclerosis.[7] In this context, the present study is designed to carry out evaluation of hypolipidemic effect of leaves of *Houttuynia cordata* Thunb. in hyperlipidaemic rats.

Houttuynia cordata Thunb. is one of the important plants of the family Saururaceae.[8] This plant is grown widely in

South-East Asian region. It's natural habitat extends up to the Assam region in India on one side and to the Java region of Indonesia on the other. *Houttuynia cordata* has different common names : Chameleon plant, Chinese lizard tail, fish mint, fishwort, heart leaf, Himalayan spinach etc. It grows naturally in moist loamy soils, shallow water and low light intensity. The leaf of this plant has an unusual taste that is often described as fishy.[9] The herb is used as a folk medicine as it has antiviral, antibacterial, immune-stimulant, diuretic, anti-cancer, and anti-inflammatory effects.[10,11] It is considered a good blood purifier. Leaves are used to cure stomach ulcers and boiled extract of rhizomes for muscular pains. The brew made of dried herbs is considered very effective for detoxification, hypertension, constipation, pulmonary tuberculosis, and diuretics.[12]

MATERIAL AND METHODS

Plant material

Houttuynia cordata Thunb. leaves were collected from Assam Medical College and Hospital campus, Dibrugarh in the month from April to August 2012, and plant materials were authenticated by Dr. L.R. Saikia, Reader, Department of Life Sciences, Dibrugarh University, Dibrugarh, Assam, India.

Preparation of the extract

Houttuynia cordata Thunb. leaves were collected and washed thoroughly and dried at room temperature separately. The dried leaves were ground into powder. A sufficient number of powdered leaves were moistened with 95% ethyl alcohol and allowed to remain for 6 hours in a percolator. When the liquid began to drop from the percolator, the orifice was closed and the content was allowed to macerate for 24 hours. After 24 hours, it was allowed to percolate slowly at a rate not exceeding 1 ml/ min and the solution was collected in Petri dishes. Alcohol was allowed to evaporate at room temperature. When the extract got completely dried, it was scrapped out, weighed, and stored.[13]

Animals

The study was carried out in healthy albino rats (6-8 months aged) of Wistar strain (*Rattus norvegicus*) of either sex of body weight between 150-200 g. Animals were procured from the Central Animal House (Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) registration number: 634/GO/Re/S/02/CPCSEA), Assam Medical College and Hospital, Dibrugarh, Assam. They were given a standard animal diet consisting of Bengal gram, wheat, maize, and carrot in sufficient quantity, and water was given ad libitum during the entire period of the experiment. The animals were housed in standard conditions with natural light and dark cycles and adequate ventilation. The study was permitted by the Institutional Animal Ethics Committee (IAEC), Assam Medical College, Dibrugarh, Assam, with approval number IAEC/AMC/02 dated 07/08/11 and conducted in accordance with the CPCSEA guidelines.

Sample collection

Under all aseptic and antiseptic measures and under ether inhalation anaesthesia, blood samples were collected from the retro-orbital sinus with the help of a capillary tube. The capillary tube was inserted at the medial canthus into the retro-orbital plexus with gentle rotation so that blood flowed into it by capillary action.[14]

Acute Toxicity Tests

The acute toxicity of Ethanolic Extract of leaves of *Houttuynia cordata* Thumb. (EELHC) was determined on female albino rats of Wistar Strain weighing 150-200g. After administration of different doses of the extract, the mortality with each dose was noted as per OECD (Organization for Economic Cooperation and Development, 2006) guidelines 425.[15]

Induction of hyperlipidemia

A high-fat diet, consisting of coconut oil and vanaspati ghee, in a ratio of 2:3 v/v at a dose of 10 ml/Kg body weight, was fed to the animals, orally, daily, in addition to the normal diet for a period of 8 weeks.[16,17]

Experimental design

A total of 20 animals of either sex weighing 150-200g were divided into four groups of five animals each and were treated as follows:

Group I (Normal Control): Received normal diet and normal saline at a dose of 10 ml/Kg/day.

Group II (Hyperlipidaemic Control): Received high-fat diet at a dose of 10 ml/Kg/day.

Group III (Hyperlipidaemic Test): Received high-fat diet at a dose of 10 ml/Kg/day and EELHC at a dose of 500 mg/Kg/day.

Group IV (Hyperlipidaemic Standard): Received high-fat diet at a dose of 10 ml/Kg/day and Simvastatin at a dose of 1.8 mg/Kg/day.

The drugs were administered once daily orally for a period of 8 weeks by intragastric feeding tube. At the end of 8 weeks, all the animals were kept fasting for 12 hours and blood samples were collected from each rat to assess the various parameters of lipid profile and antioxidant status. This fasting may hold true due to two main reasons –

1. Postprandial triglycerides remain elevated for several hours,[18]

2. Most reference values for serum lipids are established on fasting blood specimens [19]

Estimation of the biochemical parameters

Serum was separated from the blood after clotting and centrifuged for 5 minutes at 3000 rpm. The serum thus obtained was used for biochemical estimations.

The total serum cholesterol estimation was done by the method described by Allain CC *et al.* (1974) [20] using Qualigens-Diagnostics Cholesterol Kit manufactured by Sigma Diagnostics (India) Pvt. Ltd., Baroda.

Triglycerides were measured by enzyme colorimetric method as described by Fossati P *et al.* (1982) [21] using Qualigens-Diagnostics Triglyceride Reagent GPO manufactured by Sigma Diagnostics (India) Pvt. Ltd., Baroda.

HDL–Cholesterol was assayed by the method of Izzo C *et al.* (1981) [22] using Qualigens-Diagnostics HDL-cholesterol Kit manufactured by Sigma Diagnostics (India) Pvt. Ltd., Baroda.

LDL–Cholesterol was measured by using the formula of Friedewald WT *et al.* (1972). [23]

$$\text{LDL Cholesterol} = \text{Total Cholesterol} - \text{HDL Cholesterol} - \frac{\text{Triglyceride}}{5}$$

The Atherogenic Index (AI) and Percent Protection were calculated using the following formulae: [24]

$$\text{Atherogenic Index (AI)} = \frac{\text{Total Serum Cholesterol}}{\text{HDL Cholesterol}}$$

$$\text{Percent Protection} = \frac{\text{AI of hyperlipidaemic control} - \text{AI of treated group}}{\text{AI of hyperlipidaemic control}} \times 100$$

Estimation of antioxidant status

Malondialdehyde (MDA) was measured in plasma while Catalase (CAT) and Superoxide Dismutase (SOD) were measured in the erythrocytes. MDA level was estimated by the method described by Satoh K, [25] the CAT level was estimated by the method described by Beers and Sizer [26] and the SOD level was estimated by the method described by Kakkar *et al.* [27]

Statistical analysis

Statistical Analysis was done using the software Graph pad Prism version 5. All the values were expressed as mean ± SEM. The results were analyzed for statistical significance by using one-way ANOVA, followed by Dunnett's test. P values that were < 0.05 were considered significant.

RESULTS

Result of Acute Toxicity Test

There was no mortality recorded for the extracts of the leaves of *Houttuynia cordata* Thunb. among the rats up to the maximum dose of 2000 mg/Kg when administered orally. Hence, the LD50 can be said to be above 2000mg/kg.

Changes in blood lipid profile

At the end of the experiment, the hyperlipidaemic control group showed a significant ($p<0.05$) elevation of total cholesterol, triglycerides, and LDL cholesterol together with a significant ($p<0.05$) decrease in HDL Cholesterol when compared with the normal control group. Results are presented in Table 1.

In the hyperlipidaemic test group as well as in the hyperlipidaemic standard group there was a significant ($p<0.05$) reduction in total cholesterol, triglycerides, and LDL Cholesterol. HDL cholesterol was also increased significantly ($p<0.05$) in both groups.

This indicates that EELHC is effective in reducing total cholesterol, triglycerides, and LDL cholesterol and increasing HDL cholesterol. The results are presented in Table 1.

Changes in lipid peroxidation and blood antioxidant levels

At the end of the experiment, there was a significant increase ($p<0.05$) in the serum MDA level and a significant decrease ($p<0.05$) in blood CAT and SOD levels in the hyperlipidaemic control group when compared to the normal control group.

There was a significant ($p<0.05$) decrease in serum MDA levels in the hyperlipidaemic test group and Hyperlipidaemic standard group when compared to the hyperlipidaemic control group. Blood CAT and SOD levels were increased significantly in the hyperlipidaemic test group and hyperlipidaemic standard group when compared to the hyperlipidaemic control group. This indicates that EELHC decreases lipid peroxidation and increases the antioxidant enzymes in the blood. The results are presented in Table 2.

DISCUSSION

The present study was undertaken to evaluate the antihyperlipidemic and antioxidant activities of leaves of *Houttuynia cordata* Thunb.

The elevated level of total cholesterol is one of the major factors for occurrence of coronary heart disease (CHD). [28] Hyperlipidaemia, high cholesterol diet, and oxidative stress increases serum LDL levels resulting in an increased risk of development of atherosclerosis. [29]

In the hyperlipidaemic control group, the total cholesterol triglycerides and LDL cholesterol levels in the blood are

Table 1: Changes in Serum Lipid Profile

Groups	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Atherogenic index	% protection
Normal control	72.44 + 1.98	68.28 + 1.73	23.91 + 1.77	36.07 + 1.57	3.03	-
Hyperlipidaemic Control	239.38 + 2.05[a]	217.10 + 1.86[a]	11.44 + 2.03[a]	184.52 + 1.75[a]	20.92	-
Hyperlipidaemic test (EELHC)	114.71 +2.01[b]	74.07 +1.92[b]	39.29 +2.01[b]	55.22 +1.98[b]	2.92	86.04
Hyperlipidaemic standard	75.36 + 2.30[b]	59.82 + 1.79[b]	34.71 + 1.88[b]	28.69 + 1.63[b]	2.17	89.63

Values are expressed as MEAN ± SEM; (n=5).
One Way ANOVA followed by Dunnett's multiple comparison tests is done.
[a] P<0.05 when compared to the normal control group
[b] P<0.05 when compared to the hyperlipidaemic control group.

Table 2: Changes in lipid peroxidation and blood antioxidant levels

Groups	MDA (nmol/ml blood)	CAT (u/mg protein)	SOD (u/mg protein)
Normal Control	1.52+0.72	396.2+0.86	7.2+0.21
Hyperlipidaemic Control	4.34+0.04[a]	143.8+0.37[a]	4.1+0.61[a]
Hyperlipidaemic Test (EELHC)	2.58+0.68[b]	190.4+0.19[b]	5.1+0.09[b]
Hyperlipidaemic standard	1.82+0.95[b]	327.8+3.86[b]	6.9+0.17[b]

Values are expressed as MEAN + SEM; (n= 5)
One Way ANOVA followed by Dunnett's multiple comparison tests is done.
P< 0 05 when compared to the normal control group
[b] P < 0.05 when compared to the hyperlipidaemic control group

significantly (p<0.05) increased together with a decrease in HDL cholesterol level.

Elevated levels of all lipoproteins except HDL are associated with an increased risk of atherosclerosis.[30]

The hyperlipidaemic test group fed with a high-fat diet and EELHC showed significant (p<0.05) decreases in the total cholesterol, triglyceride, and LDL Cholesterol which is almost comparable to the Standard group fed with a high-fat diet and simvastatin.

On the other hand, HDL cholesterol level is significantly (p<0.05) increased in the hyperlipidaemic test group as well as in the standard group.

HDL cholesterol is referred to as the 'good cholesterol' because HDL is involved in the transport of cholesterol from peripheral tissues to the liver and thereby reducing the amount stored in the tissue and the possibility of developing atherosclerotic plaques.[30]

Atherogenic index (AI) calculated as the ratio between total cholesterol and HDL cholesterol is used as a marker to assess the susceptibility of atherogenesis.[31] It is an important indicator of CHD risks at both high and low serum cholesterol levels.[32] When compared with the hyperlipidaemic control group there is a significant decrease in the atherogenic index in the group fed with EELHC (2.92), which is almost comparable to the standard (2.17).

The persistence of a hypercholesterolemic state causes enhanced oxidative stress, leading to the development of atherosclerosis, coronary artery disease (CAD), and other complications of obesity.[33]

Hypercholesterolemia increases the levels of the lipid peroxidation product Malondialdehyde. The increase in Malondialdehyde (MDA) levels in the liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals.[34] Oxygen free radicals have been implicated in the development of hyperlipidaemic atherosclerosis. SOD and catalase mimic the effective

detoxification of Oxygen free radical and Hydrogen peroxide among these, SOD convert the highly toxic superoxide (O_2^-) to less toxic hydrogen peroxide and O_2, this is the first line of defense to protect the cells from the injurious effects of superoxide. Hydrogen peroxide produced by superoxidedismutase is further metabolized by catalase.[35] The hyperlipidaemic test group fed with HFD showed a significant increase in MDA and a decrease in CAT and SOD, indicating increased lipid peroxidation and decreased antioxidant enzyme levels. The hyperlipidaemic test group which was fed with EELHC showed a significant decrease in MDA and an increase in CAT and SOD.

It is well known that flavonoids and polyphenols have been shown to have hypolipidemic and antioxidant activity.[36] Flavonoids present in medicinal plants protect LDL from oxidation thereby decreasing atherosclerotic plaques. They are reported to significantly increase superoxide dismutase and catalase activity.[29] Several naturally occurring active components are found in *Houttuynia cordata* Thumb. Leaves may be the reason behind its antihyperlipidemic and antioxidant activity.

CONCLUSION

The present study suggests that ethanolic extract of leaves of *Houttuynia cordata* Thunb. has antihyperlipidemic and antioxidant activities. This was evident from decreased lipid levels with an increase in HDL even with the daily administration of the atherogenic diet and decreased MDA with an increase in CAT and SOD levels respectively. *Houttuynia cordata* Thunb. leaves reduce oxidative stress by free radical scavenging and protecting against lipid peroxidation and are also able to manage hyperlipidemia by decreasing serum levels of cholesterol, triglycerides, and LDL. The probable antihyperlipidemic mechanism may be due to decreased absorption of exogenous cholesterol and increased conversion of endogenous cholesterol to bile acids. Further pharmacological and biochemical investigations are needed to determine the precise mechanism and site of action and the active constituents involved so that these herbal drugs can be used as a safer alternative to synthetic drugs.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

REFERENCES

1. Ross R. Atherosclerosis–an inflammatory disease. *N Engl J Med*. 1999;340(2):115–126. Available from: https://doi.org/10.1056/NEJM199901143400207.
2. Shrivastava R, Solanki SS, Tomar V, Garud N, Kannojia P. Comparative Evaluation of polyherbal combination for hypolipidemic activity. *Indian Journal of Pharmaceutical Sciences and Drug Research*. 2009;1(1):9–12. Available from: https://doi.org/10.25004/IJPSDR.2009.010103.
3. Ashakumary L, Vijayammal PL. Additive effect of alcohol and nicotine on lipid metabolism in rats. *Indian J Exp Biol*. 1993;31(3):270–274.
4. Sudha SS, Karthic E, Naveen, Rengaramanujam J. Antihyperlipidaemic activity of Spirulinaplatensis in Triton x-100 induced hyperlipidaemic rats. *Hygeia: Journal for drugs and medicines*. 2011;3(2):32–37. Available from: http://www.hygeiajournal.com/Downloads/2337269785.SUDHA.pdf.
5. Pandya N, Santani D, Jain S. Antioxidant activity of ezetimibe in hypercholesterolemic rats. *Indian Journal of Pharmacology*. 2006;38(3):205–206. Available from: https://doi.org/10.4103/0253-7613.25811.
6. Speight TM. Avery's Drug Treatment Principles and Practice of Clinical Pharmacology and Therapeutics. 3rd ed. ADIS Press Ltd. 1987.
7. Ikeda I, Sugano M. Inhibition of cholesterol absorption by plant sterols for mass intervention. *Curr Opin Lipidol*. 1998;9(6):527–531. Available from: https://doi.org/10.1097/00041433-199812000-00003.
8. Liang HX. On the evolution and distribution in Sauruaceae. *Acta Bot Yunnan*. 1995;17(3):1–3. Available from: https://journal.kib.ac.cn/EN/Y1995/V17/I03/1.
9. Ravindran PN. The Encyclopedia of Herbs and Spices. vol. 1. Wallingford, UK. CAB International. 2017;p. 233–237. Available from: https://doi.org/10.1079/9781780643151.0000.
10. Yoshinoh IN, Nabae K, Doi Y, Tamano S, Ogawa K, Shirai T. Thirteen-week oral toxicity study of Dokudami extract (Houttuynia cordata Thunb.) in F344/ DuCrj rats. *J Toxicol Pathol*. 2005;18:175–182. Available from: https://doi.org/10.1293/tox.18.175.
11. Lu HM, Liang YZ, Wu XJ, Qiu P. Tentative fingerprint-efficacy study of Houttuynia cordata injection in quality control of traditional Chinese medicine. *Chem Pharm Bull (Tokyo)*. 2006;54(5):725–730. Available from: https://doi.org/10.1248/cpb.54.725.
12. Bhattacharyya N, Sarma S. Assessment of availability, ecological feature, and habitat preference of the medicinal herb Houttuynia cordata Thunb in the Brahmaputra Valley of Assam, India. *Environ Monit Assess*. 2010;160(1-4):277–287. Available from: https://doi.org/10.1007/s10661-008-0694-7.
13. Gennaro AR. Solution, Emulsion, Suspension, and Extracts. In: Remington: The Science and Practice of Pharmacy. UK. Lippincott Williams & Wilkins.. 2006;p. 1495–1523.
14. Ghosh MN. Fundamentals of Experimental Pharmacology. 6th ed. Kolkata. Hilton and Company. 2015.
15. OECD. Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure. In: OECD Guidelines for the Testing of Chemicals, Section 4;vol. 4. Paris. OECD Publishing. 2022. Available from: https://doi.org/10.1787/9789264071049-en.
16. Shyamala MP, Venukumar MR, Latha MS. Antioxidant potential of the Syzygium aromaticum (Gaertn.) Linn. (cloves) in rats fed with high-fat diet. *Indian Journal of Pharmacology*. 2003;35(2):99–103. Available from: https://www.ijp-online.com/article.asp?issn=0253-7613;year=2003;volume=35;issue=2;spage=99;epage=103;aulast=Shyamala;type=0.
17. Jain GC, Jhalani S, Agarwal S, Jain K. Hypolipidemic and Antiatherosclerotic Effect of Leptadenia pyrotechnica Extract in Cholesterol Fed Rabbits. *Asian J Exp Sci*. 2007;21(1):115–122. Available from: http://ajesjournal.com/PDFs/07-1/13-G%20C%20Jain.pdf.
18. Campose H, Khoo C, Sacks FM. Diurnal and acute pattern of postprandial apolipoprotein B- 48 in VLDL, IDL and LDL from normolipidaemic human. *Atherosclerosis*. 2005;181(2):345–351. Available from: https://doi.org/10.1016/j.atherosclerosis.2004.12.045.
19. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third report of the National Cholesterol Education Program (NCEP) Expert panel on detection, evaluation and treatment of high blood cholesterol in adults (adult treatment panel III) final report. *Circulation*. 2002;106(25):3143–3421. Available from: https://doi.org/10.1161/circ.106.25.3143.
20. Mahdi AA, Chandra A, Singh RK, Shukla S, Mishra LC, Ahmad S. Effect of herbal hypoglycemic agents on oxidative stress and antioxidant status in diabetic rats. *Indian J Clin Biochem*. 2003;18(2):8–

15. Available from: https://doi.org/10.1007/BF02867361.
21. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical Chemistry*. 1982;28(10):2077–2080. Available from: https://pubmed.ncbi.nlm.nih.gov/6812986/.
22. Izzo C, Grillo F, Murador E. Improved method for determination of high-density-lipoprotein cholesterol I. Isolation of high-density lipoproteins by use of polyethylene glycol 6000. *Clinical Chemistry*. 1981;27(3):371–374. Available from: https://pubmed.ncbi.nlm.nih.gov/7471383/.
23. 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. Available from: https://pubmed.ncbi.nlm.nih.gov/4337382/.
24. Mazumder PM, Farswan M, Parcha V. Effect of an isolated active compound (Cg-1) of Cassia glauca leaf on blood glucose, lipid profile, and atherogenic index in diabetic rats. *Indian J Pharmacol*. 2009;41(4):182–186. Available from: https://doi.org/10.4103/0253-7613.56076.
25. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin Chim Acta*. 1978;90(1):37–43. Available from: https://doi.org/10.1016/0009-8981(78)90081-5.
26. Beers RF, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *Journal of Biological Chemistry*. 1952;195(1):133–140. Available from: https://pubmed.ncbi.nlm.nih.gov/14938361/.
27. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Indian J BiochemBiophys*. 1984;21(2):133–140. Available from: https://pubmed.ncbi.nlm.nih.gov/6490072/.
28. Gangireddy RR, Challa SR, Rao CV. Comparative and combined studies on antidiabetic, antioxidant, and hypolipidaemic effects of two plant extracts in STZ-induced diabetic rats. *Asian J Trad Med*. 2011;6(6):231–241.
29. Visavadiya NP, Narasimhacharya A. Hypolipidaemic and antioxidant activity of Asparagus racemosus in hypercholesteraemic rats. *Indian J Pharmacol*. 2005;37(6):376–380. Available from: https://www.ijponline.com/text.asp?2005/37/6/376/19074.
30. Saikia H, Lama A. Effect of Leaves on Serum Lipids in Albino Rats Fed with High Fat Diet. *International Journal of Pharmaceutical Sciences and Drug Research*. 2011;3(2):141–145. Available from: http://www.ijpsdr.com/index.php/ijpsdr/article/view/266.
31. Parasuraman S, Kumar EP, Kumar A, Emerson S. Antihyperlipidemic effect of Triglize, a polyherbal formulation. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2010;2(3):118–122. Available from: https://innovareacademics.in/journal/ijpps/Vol2Issue3/607.pdf.
32. Lakhsmi BVS, Neelima N, Kasthuri N, Umarani V, Sudhakar M. Anti-hyperlipidaemic activity of Bauhunia purpura extracts in hypercholesterolaemic albino rats. *International Journal of Pharmtech Research*. 2011;3(3):1265–1272. Available from: https://sphinxsai.com/Vol.3No.3/pharm/pdf/PT=08(1265-1269)JS11.pdf.
33. Dhamija HK, Chauhan AS. Herbs: Now in arena of hyperlipidemia. *Der Pharmacia Sinica*. 2011;2(3):51–59.
34. Dash DK, Yeligar VC, Nayak SS, Ghosh T, Rajalingam R, Sengupta P, et al. Evaluation of hepatoprotective and antioxidant activity of Ichnocarpus frutescens (Linn.) R.Br. on paracetamol-induced hepatotoxicity in rats. *Tropical Journal of Pharmaceutical Research*. 2007;6(3):755–765. Available from: https://doi.org/10.4314/tjpr.v6i3.14656.
35. Satishchandra A, Sumithra M. Synergistic effect of Mimusopselengiand Moringa oleifera onhigh-fat diet induced atheroma in rats. *International Journal of Advances in Pharmaceutical Research*. 2011;2(6):293–300.
36. Yu YMM, Wu CHH, Tseng YHH, Tsai CE, Chang WC. Antioxidative and Hypolipidemic Effects of Barley Leaf Essence in a Rabbit Model of Atherosclerosis. *Japanese Journal of Pharmacology*. 2002;89(2):142–148. Available from: https://doi.org/10.1254/jjp.89.142.