IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 9, Issue 4 Ver. V (Jul -Aug. 2014), PP 55-59
www.iosrjournals.org

Preclinical Lipid profile studies of a classical Ayurvedic preparation "Kutajarista" after chronic administration to male Sprague-Dawley rats

Kaniz Fatema, Md. Rakib Hasan, Md. Mahmudur Rahman Mamun, Projjal Kanti Biswas, Shuvagata Kahali, Paritosh Chakma, Mohammed Motaher Hossain Chowdhury and M. S. K. Choudhuri.
Department of Pharmacy, Jahangirnagar University, Savar, Dhaka - 1342, Bangladesh.

Abstract: *Kutajarista (KTJ) is an Ayurvedic preparation used as a traditional medicine in the South Asian countries for the treatment of diarrhoea, dysentery and irritable bowel syndrome. The effect of chronic administration of KTJ on the serum lipid profile was studied in this experiment. After 28 days of chronic administration of the KTJ preparation to the male Sprague-Dawley rats at a dose of 40 ml/kg the following biochemical changes were noted. There was a statistically very highly significant (p=0.001) increase in serum LDL-C level whereas a significant increase was noted in case of total cholesterol (TC) level (p=0.013). A significant (p=0.039) decrease was noticed in HDL-C level but in case of Non-HDL-C level, a highly significant (p=0.002) increase was noticed. Besides, a statistically very highly significant (p=0.001) decrease was noted in case of serum triglyceride (TG) and VLDL-C level; thus leading to a statistically highly significant increase (p=0.004) in Cardiac Risk Ratio (CRR) (TC/HDL-C) and Atherogenic Coefficient (AC) ((TC -HDL-C)/HDL-C), a statistically very highly significant (p=0.001) increase in Castelli's Risk Index-II (CRI- II) (LDL-C/HDL-C) but a statistically very highly significant decrease (p=0.001) in case of Atherogenic Index of Plasma (AIP) ((log (TG/HDL-C)).*

Keywords: *Kutajarista, Lipid profile, Cardiac Risk Ratio, Atherogenic Index of Plasma, Atherogenic Coefficient.*

I. Introduction

Ayurvedic medicine is the oldest holistic medicines system and is derived from a Sanskrit world 'ayus' (life) and 'veda' (knowledge) which means the "science of life". Ayurveda is the most ancient science of healing which improves longevity. It has influenced many of the older traditional methods of healing including Tibetan, Chinese and Greek medicine. Hence, Ayurveda is considered by many as the "mother of healing". Ayurveda can be defined as a system, which uses the inherent principles of nature, to help maintain health in a person by keeping the individual's body, mind and spirit in perfect equilibrium with nature. Ayurveda represents the ancient Indian art of healing in which the human body is not considered just as a mass of organs, systems and tissues; but the complex mechanism of myriad functions taking place, both at physical and mental level.

Ayurveda originated in India more than 3,000 years ago and remains as one of the country's traditional health care systems. Its concepts about health and disease promote the use of herbal compounds, special diets, and other unique health practices. Ayurvedic medicines are regarded as a part of complementary and alternative medicine recognized by World Health Organization (WHO), National Institutes of Health (NIH) and others [1]. Ayurvedic medicine uses a variety of products and practices. The safety profile of these drugs has not been fully investigated. It is also not clear, whether these preparations might interact with other drugs or diagnostic tests. The present study was undertaken to explore the effect of the drug in the lipid profile of rat serum after chronic administration of the drug.

Kutajarishta (KTJ) is a liquid Ayurvedic medicine, used in the treatment of diarrhea, dysentery or blood dysentery fever, irritable bowel syndrome, Crohn's disease, ulcerative colitis mal-absorption syndrome or sprue etc. [3-7]. It contains 5 – 10 % of self-generated alcohol in it. This self-generated alcohol and the water present in the product acts as a media to deliver water and alcohol soluble active herbal components to the body. Kutajarista is included (pages 81) in the Bangladesh National Formulary of Ayurvedic Medicine 1992 (Approved by the Government of Bangladesh vide Ministry of Health and Family Welfare Memo No. Health-1/Unani-2/89/ (Part-1) 116 dated 3-6-1991).

Table-1: Name of the ingredients/herbs used in the preparation of "Kutajarishta" (KTJ)

Sanskrit name	Botanical or scientific name of Plants	Parts used	Quantity used
Kutaja	*Holarrhena antidysenterica*	Stem bark or Root	4.80 kg
Mrdvika (Draksa)	*Vitis vinifera*	Dry fruit	2.40 kg
Madhuka pushpa	*Madhuca longifolia*	Flower	0.48 kg
Kashmari (Ghambari)	*Gmelina arborea*	Stem bark	0.48 kg
Water for decoction Reduced to	-	-	49.152 L 12.288 L
Guda (Jaggery)	-	-	4.80 kg
Dhataki	*Woodfordia fruticosa*	Flower	0.96 kg

II. Materials And Methods

Drugs, Chemicals and Reagents

For the toxicological study, Kutajarishta (KTJ) was collected from Sri Kundeswari Aushadhalaya Limited, Chittagong. Ketamine injection was purchased from ACI Limited, Bangladesh. All other reagents, assay kits and chemicals used in this work were purchased from Human GmbH, Wiesbaden, Germany.

Experimental Animals

Six to eight-week old male Sprague-Dawley rats bred and maintained at the animal house of the Department of Pharmacy, Jahangirnagar University, were used in the toxicological experiment. These animals were apparently healthy and weighed 50-70 g. The animals were housed in a well-ventilated clean experimental animal house under constant environmental and adequate nutritional conditions throughout the period of the experiment. They were fed with rat chow prepared according to the formula developed at Bangladesh Council of Scientific and Industrial Research (BCSIR). Water was provided *ad libitum* and the animals maintained at 12 h day and 12 h night cycle. All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals approved by Ethical Review Committee, Faculty of Life Sciences, Department of Pharmacy, Jahangirnagar University.

Experimental Design

Acute toxicity study

The acute oral toxicity test was performed following the guidelines of Organization for Economic Co-operation and Development (OECD) for testing of chemicals with minor modifications (OECD Guideline 425) [8]. Sixteen female mice (non-pregnant, 35-40 g body weight) were divided into four groups of four animals each. Different doses (50 ml/kg, 60 ml/kg, 70 ml/kg and 80 ml/kg) of experimental drug (KTJ) were administered by stomach tube. The dose was divided into two fractions and given within 12 hours. Then all the experimental animals were observed for mortality and clinical signs of toxicity (general behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes and changes in skin and fur texture) at 1, 2, 3 and 4 hours and thereafter once a day for the next three days following KTJ administration.

Chronic toxicity studies

Prior to the experiment, rats were randomly divided into 2 groups of 8 animals each. One group was treated with KTJ and another was used as a control. The control animals were administered with distilled water only as per the same volume as the drug treated group for 28 days. For all the pharmacological studies the drug was administered per oral route at a dose of 40 ml/Kg body weight [9]. After acclimatization, Ayurvedic medicinal preparation was administered to the rats by intra-gastric syringe between the 10 am to 12 am daily throughout the study period. All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals. The experiment animals were marked carefully on the ear which helped to identify a particular animal. By using identification mark, responses were noted separately for a particular period prior to and after the administration [10].

Blood Samples Collection and Preparation of Serum

At the end of 28 days treatment, after 18 h fasting, blood samples were collected from post vena cava of the rats anaesthetizing with Ketamine (500 mg/kg body, intra peritoneal) and transferred into tubes immediately [11]. Blood was let to clot and was then centrifuged at 4,000 *g* for 10 min using bench top centrifuge (MSE Minor, England). The supernatant serum samples were collected using dry Pasteur pipette and stored in the refrigerator for further analyses. All analyses were completed within 12 h of sample collection [12].

Determination of Lipid Profile Parameters

Lipid profile studies involved analysis of parameters such as triglyceride (TG) level determined by GPO-PAP method [13]; total cholesterol (TC) level determined by CHOD-PAP method [14]; LDL-cholesterol level determined by CHOD-PAP method [15]; HDL-cholesterol level determined by CHOD-PAP method [16].

The absorbance of all the tests was determined using Humalyzer, Model No-3500 (Human GmbH, Wiesbaden, Germany).

Serum VLDL and LDL cholesterol concentrations were calculated using the Friedewald equation [17] as follows:

i. **LDL cholesterol** (mg/dl) = Total cholesterol – (HDL cholesterol −Triglyceride / 5)

ii. **VLDL cholesterol** (mg/dl) = Triglyceride / 5.

While the serum non-HDL cholesterol concentration was determined as reported by Brunzell [18]:

Non-HDL cholesterol = Total cholesterol − HDL cholesterol.

The atherogenic indices were calculated as follows:

Cardiac Risk Ratio (CRR) =TC/HDL-C [19].

Castelli's Risk Index (CRI-II) = LDL-C/HDL-C [20].

Atherogenic Coefficient (AC) = (TC -HDL-C)/HDL-C [21].

Atherogenic Index of Plasma (AIP) = log (TG/HDL-C) [22].

(Note: for calculation of atherogenic indices mg/dl values of TC, HDL-C, LDL-C and TG were converted into mmol/L)

Statistical Analysis

The data were analyzed using independent sample t-test with the help of SPSS (Statistical Package for Social Science) Statistics 11.5 package (SPSS Inc., Chicago Ill). All values are expressed as mean ± SEM (Standard error of the mean) and $p<0.05$, $p<0.01$, $p<0.001$ was taken as the level of significance.

III. Results

Acute toxicity study

The drug (KTJ) administered up to a high dose of 80 ml/kg produced no mortality. Thus the LD_{50} value was found to be greater than 80 ml/kg body weight. The animals did not manifest any sign of restlessness, respiratory distress, general irritation or convulsion. Since KTJ is in the clinical use for treatment of diarrhea, dysentery, irritable bowel syndrome for many years, a limit test was performed in acute oral toxicity study. According to the OECD test guideline 425 when there is information in support of low or non-toxicity and immortality nature of the test material, then the limit test at the highest starting dose level (80 ml/kg body weight) was conducted. There were no mortality and toxicity signs observed at 80 ml/kg body weight. Therefore, it can be concluded that KTJ when administered at single dose is non-toxic and can be used safely in oral formulations.

Chronic Lipid Profile Studies

Effect of KTJ on lipid profile of male rats

In the male rats there was noticeable increase in the total cholesterol (TC), LDL-C, Non-HDL-C level and a very highly significant decrease in the triglyceride (TG) and VLDL-C level in the serum. A significant decrease was noticed in case of HDL-C level.

After chronic administration of KTJ the total cholesterol level was (22.13%, p=0.013) increased in male rats group which was statistically highly significant. In this investigation, a statistically highly significant (p=0.002) increase was observed in case of Non-HDL-C (67.93%) level and a very highly significant increase (p=0.001) in LDL-C (194.06%) level in the KTJ treated male rats in comparison to control. A statistically very highly significant (p=0.001) decrease was observed in case of triglyceride (52.78%), and VLDL-C (52.78%) level and a significant (0.039) decrease in case of HDL-C level (16.87%) in the KTJ treated male rats. All the results are presented in Table 2.

Table 2: Effect of KTJ on lipid profile of rat serum.

Parameters	Control	KTJ	p values	% Change
Triglycerides (TG)	74.13±6.07	35.00±2.66	0.001	↓52.78 %
Total Cholesterol (TC)	57.63±2.92	70.38±3.39	0.013	↑22.13 %
VLDL-C	14.83±1.21	7.00±0.53	0.001	↓52.78 %
LDL-C	12.63±2.85	37.13±3.67	0.001	↑194.06 %
HDL-C	31.13±1.56	25.88±1.69	0.039	↓16.87 %
Non HDL-C	25.50±3.09	44.50±3.65	0.002	↑67.93%

Effect of KTJ on atherogenic indices of male rats

In this study, KTJ augmented almost all the atherogenic indices except Atherogenic Index of Plasma (AIP). The increase in Cardiac Risk Ratio (CRR) (49.66% increase) and Atherogenic Coefficient (AC)

(106.23% increase) was statistically highly significant (p=0.004). A statistically very highly significant (p=0.001) increase in case of Castelli's Risk Index (CRI-II) (265.31% increase) and a statistically very highly significant decrease in case of Atherogenic Index of Plasma (AIP) (2663.33%) were noticed. All the results are presented in Table 3.

Table 3: Effect of KTJ on Atherogenic Indices of rat serum.

Parameters	Control	KTJ	p values	% Change
CRR	1.880±0.118	2.81 ± 0.245	0.004	↑49.66 %
CRI-II	0.417±0.099	1.52 ± 0.24	0.001	↑265.31 %
AC	0.878±0.118	1.81 ± 0.245	0.004	↑106.23 %
AIP	0.009±0.045	-0.231 ± 0.029	0.001	↓2663.33%

IV. Discussion

Effect of KTJ on lipid profile of male rats

High serum total cholesterol level is a familiar and well-known risk factor for developing atherosclerosis and other cardiovascular diseases [23]. Therefore KTJ may have been responsible for the hyper-cholesterolemic effect, observed in this study. Numerous studies have presented that non-HDL cholesterol is a better predictor of cardiovascular disease risk than is LDL cholesterol [24-25]. Therefore, the significantly higher plasma non-HDL cholesterol levels observed in the treated groups is indicative of the ability of the drug to increase cardiovascular risk. High level of plasma LDL and VLDL cholesterol are risk factors for cardiovascular disease [26-27] and often accompany hypertension [28] and obesity [29]. In this study, significantly higher plasma LDL and significantly lower VLDL cholesterol levels were observed in the animals treated with KTJ.

A high plasma triglyceride level is both an independent and synergistic risk factor for cardiovascular diseases [30] and is often related with hypertension [31], obesity and diabetes mellitus [32]. In this study, significantly lower serum triglyceride level was observed in the animals treated with KTJ. Therefore KTJ may have been responsible for the hypo-triglyceridemic effect.

Reduced serum HDL cholesterol is a risk factor for cardio-vascular diseases [33] and is often found in hypertension [28, 31]. So, in the present study, the low serum HDL cholesterol level, recorded for the treated groups is suggestive of the cardio-toxic effect of the drug.

Effect of KTJ on atherogenic indices of male rats

In this study, KTJ augmented almost all the atherogenic indices except AIP. The increase in Cardiac Risk Ratio (CRR) and Atherogenic Coefficient (AC) was statistically highly significant. The increase in Castelli's Risk Index (CRI-II) and the decrease in Atherogenic Index of Plasma (AIP) were statistically very highly significant. Atherogenic indices are strong indicators of the risk of heart disease: the higher the value, the higher the risk of developing cardiovascular problems and vice versa [20-21].

V. Conclusion

From the above experiment it can be concluded that KTJ should not be administered chronically at a higher dose as it increase Total Cholesterol (TC), LDL-C, Non-HDL-C, almost all atherogenic indices except AIP and decrease HDL-C level. Further studies should be done by reducing the administered dose. Thus Kutajarishta is to be taken only at a dosage of 12–24 mL once or twice a day usually advised after food. If needed, it can be mixed with equal quantity of water.

Acknowledgment

The authors are thankful to Focused Research on Ayurvedic Medicine and Education (F.R.A.M.E) Laboratory, Department of Pharmacy and all faculty members and the technical staffs of the Department of Pharmacy, Jahangirnagar University for their kind co-operation. We would express our special thanks to Mr Shafiqul Islam for ensuring a constant supply of animals followed by proper maintenance and care of these animals during all throughout the experimental period.

References

[1] Valiathan M. S. Ayurveda: putting the house in order. Current Science (Indian Academy of Sciences) 2006, 90 (1): 5–6.
[2] Keen R. W., Deacon A. C., Delves H. T., Moreton J. A., Frost P. G. Indian herbal remedies for diabetes as a cause of lead poisoning, Postgrad Med J. 1994, 70:113–4.
[3] Dash B. Diagnosis and Treatment of diseases in Ayurveda (Based on Ayurveda Saukhyam of Todarananda). Vol I-V. Concept Publishing Company, New Delhi, 1984, p- 2578 p.
[4] Dastur J. F. Everybody's guide to Ayurvedic medicine - A repertory of therapeutic prescriptions based on the indigenous system of India.Taraporevala Sons and Co., Bombay.1960, p- 212.
[5] Mishra, Chandra L. Scientific Basis for Ayurvedic Therapies. CRC Press, Reprint, xxii, 2010, p- 626.

[6] Nadkarni A. K. Indian Materia Medica, Vol. 1. Popular Book Depot, Bombay, India, 1976.
[7] Verma H. K. Comprehensive Book of Ayurvedic Medicine for General Practitioners with Annonated Key References Vol I (Based on Modern Diagnosis and Ayurvedic Treatment) Kalyani Publishers.New Delhi, 1991, p- 196.
[8] OECD Guideline (425) for the testing of chemicals, Guidance document on acute oral toxicity, Environmental Health and Safety Monograph Series on Testing and Assessment, 2000.
[9] Gad S. C. An Approach to the Design and Analysis of Screening Studies in Toxicology, Intl J Tox. 1988, 7: (2): 127-138.
[10] Stevens K. R. and Gallo M. A. Practical consideration in the conduct of chronic toxicity studies, Principles and Methods of Toxicology, 2nd ed. Chap. VIII, 1989.
[11] Ringler H. and Dabich L. Hematology and clinical biochemistry. In: The Laboratory Rat Biology and Disease [Baker HL ed].American College of Laboratory Animal Medicine Series Academic Press, 1979.
[12] Wolford S. T., Schoer R. A., Gohs F. X., Gallo P. P. Reference range database for serum chemistry and haematology values in laboratory animals. J Tox Environ Hlth. 1986, 18: 161-88.
[13] Cole T. G., Klotzsch S. G., Namara M. C. Measurement of triglyceride concentration. In: Rifai, N., Warnick, G.R., Domiminiczak, M. H., Eds. Handbook of lipoprotein testing. AACC Press, Washington. 1997, 115-26.
[14] Richmond W. Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clin Chem. 1973, 19: 1350-1356.
[15] Okada M., Matsui H., Ito Y., Fujiwara A., Inano K. Low-density lipoprotein cholesterol can be chemically measured: a new superior method. J Lab Clin Med. 1998, 132(3):195-201.
[16] Henry R. J., Winkleman J. W., Cannon D. C. Clinical Chemistry-Principles and Technics. 2nd ed. New York: Harper & Row Publishers, 1974.
[17] Friedewald W. T., Levy R. I., Friedrickson D. S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry 1972, 18: 499–502.
[18] Brunzell J. D., Davidson M., Furberg C. D., Goldberg R. D., Howard B. V., Stein J. H., Witztum J. L. Lipoprotein management in patients with cardiometabolic risk: consensus conference report from the American Diabetes Association and the American College of Cardiology Foundation. Journal of the American College of Cardiology 2008, 51: 1512–1524.
[19] Martirosyan D. M., Miroshnichenko L. A., Kulokawa S. N., Pogojeva A. V. and Zoloedov V. I. Amaranth oil application for heart disease and hypertension. Lipids Health Dis. 2007, 6:1.
[20] Castelli W. P., Abbott R. D., McNamara P. M. Summary estimates of cholesterol used to predict coronary heart disease. Circulation 1983, 67(4): 730-734.
[21] Brehm A., Pfeiler G., Pacini G., Vierhapper H., Roden M. Relationship between Serum Lipoprotein Ratios and Insulin Resistance in Obesity. Clin. Chem. 2004, 50: 2316-2322.
[22] Dobiasova M. Atherogenic Index of Plasma [log (triglyceride/HDL-Cholesterol)]: Theoretical and Practical Implications. Clin. Chem. 2004, 50: 1113-1115.
[23] Ademuyiwa O., Ugbaja R. N., Idumebor F., Adebawo O. Plasma lipid profiles and risk of cardiovascular disease in occupational lead exposure in Abeokuta, Nigeria.Lipids in Health and Disease 2005, 4: 19.
[24] Liu J., Sempos C., Donahue R., Dorn J., Trevisan M., Grundy S. M. Joint distribution of non-HDL and LDL cholesterol and coronary heart disease risk prediction among individuals with and without diabetes. Diabetes Care 2005, 28: 1916–1921.
[25] Pischon T., Girman C. J., Sacks F. M., Rifai N., Stampfer M. J., Rimm E. B. Non-high density lipoprotein cholesterol and apolipoprotein B in the prediction of coronary heart disease in men.Circulation 2005, 112: 3375–3383.
[26] Lichtenstein A. H., Appel L. J., Brands M., Carnethon M., Daniels S., Franch H. A., Franklin B., Kris-Etherton P., Harris W.S., Howard B., Karanja N., Lefevre M., Rudel L., Sacks F., Van Horn L., Winston M., Wylie-Rosett J. Summary of American heart association diet and lifestyle recommendations revision. Arteriosclerosis, Thrombosis and Vascular Biology 2006a, 26: 2186–2191.
[27] Lichtenstein A. H., Appel L. J., Brands M., Carnethon M., Daniels S., Franklin B., Kris-Etherton P., Harris W. S., Howard B., Karanja N., Lefevre M., Rudel L., Sacks F., Van Horn L., Winston M., Wylie-Rosett J., Franch H.A. Diet and lifestyle recommendations revision.A scientific statement from the American Heart Association Nutrition Committee. Circulation 2006b, 114: 82–96.
[28] Shepherd J. Identifying patients at risk for coronary heart disease: treatment implications. European Heart Journal 1998, 19: 1776–1783.
[29] Krauss R. M., Blanche P. J., Rawlings R. S., Fernstrom H. S., Williams P. T. Separate effects of reduced carbohydrate intake and weight loss on atherogenic dyslipidemia. American Journal of Clinical Nutrition 2006, 83: 1025–1031.
[30] McBride P. E. Triglycerides and risk for coronary heart disease. Journal of American Medical Association 2007, 298: 336–338.
[31] Zicha J., Kunes J., Devynck M. A., Abnormalities of membrane function and lipid metabolism in hypertension: a review. American Journal of Hypertension 1999, 12: 315–331.
[32] Shen G. X. Lipid disorders in diabetes mellitus and current management. Current Pharmaceutical Analysis 2007, 3: 17–24.
[33] Lewis G. F., Rader D. J. New insights into the regulation of HDL metabolism and reverse cholesterol transport. Circulation Research 2005, 96: 1221–1232.