IAMJ

Research Article *International Ayurvedic Medical Journal* *ISSN:2320 5091*

STUDIES OF LIPID PROFILE, LIVER AND KIDNEY FUNCTION PARAMETERS OF RAT PLASMA AFTER THE ADMINISTRATION OF *ARKADI KVATHA CURNA*

Md. Mamun Al-Amin[1]*, Sophia Hossain[2], Mohammad Shohel[1], Safayat Mahmud[3]

[1]Department of Pharmacy, North South University, Bashunndhara, Dhaka, Bangladesh
[2]Department of Pharmacy, University of Development Alternative, Dhaka, Bangladesh
[3]Unimed Unihealth, Dhaka, Bangladesh

ABSTRACT

Arkadi Kvatha Curna (AKC), a traditional Ayurvedic preparation used in puerperal disorders was carried out a biochemical evaluation in rodents (rats) for its toxicological characteristic after chronic administrations for consecutive 41 days. *AKC* was administered for 46 days orally to albino rat of both sexes. Animals were fasted for 18 hours after the last administration of *AKC*. Biochemical parameters such as Triglyceride, LDL, VLDL, HDL, Total cholesterol, Total Protein, Albumin, Bilirubin, Urea and Uric Acid amount in the plasma were measured. Serum protein & Albumin contents were significantly ($p<0.05$) increased. Triglyceride and Urea level were decreased significantly ($p<0.05$). Bilirubin contents were decreased significantly ($p<0.05$) in male rats. Serum Creatinine contents in the plasma were increased significantly ($p<0.05$). The present study confirms that AKC might have an important role for those who have hyper-triglyceridemia, liver and kidney disorder as it improves Trilgyceride, Bilirubin, Creatinine and Urea levels.

Keywords: *Arkadi Kvatha Curna (AKC)*, Biochemical Study, Toxicology

INTRODUCTION

Nature is providing the largest number of pharmacological agents over the years. This practice is going on for thousand of years.[1] Hence traditional medicine like Ayurvedic preparation is still remains a popular practice in the subcontinent including India, Sri Lanka and Bangladesh.[2] Ayurvedic medicines have a wide access to the large number of population in these countries. The acceptance of these medicines is increased due to its integrative approach for the prevention and treatment of disease through natural remedies. Traditional people are getting the benefits of this practice from ancient time. But, the uses and the safety profile of all the Ayurvedic medicines are not ensured scientifically.[3-5] Moreover, the conflict between traditional medicines and allopathic medicine are needed to be addressed scientifically in the in vivo and in vitro model. Ayurvedic preparation such as *Arkadi Kvatha Curna* (*AKC*) is a popular medicine used in puerperal disorders (*Sutikaroga*). Basically, *Arkadi Kvatha Curna* is a preparation of root *Calotropis gigantea* (*Arka*) with some other medicinal plants (Table 1). *AKC* is included in the Bangladesh National Formulary of Ayurvedic Medicine 1992.[6] Bangladesh National Formulary of Ayurvedic Medicine is compiled by the National Unani and Ayurvedic Formulary Committee and published by the Ban-

gladesh Board of Unani and Ayurvedic Systems of Medicine, 38 under the authority vested in the Board vide section 13(j) of the Bangladesh Unani and Ayurvedic practitioners Ordinance, 1983 in collaboration with the World Health Organization.

The principle components of *AKC* is *Calotropis gigantea* which has many effects such as, hypoglycemic, [7] hepatoprotective[8] and anti-inflammatory activity.[9, 10]

Table 1: Ingredients of *Arkadi Kvatha Curna* (*AKC*)

Name of Plants	Used parts	Botanical Name	Family	Quantity
Arka	Root	*Calotropis gigantea*	Asclepiadeceae	1 Part
Devadaru	Heartwood	*Cedrus deodara*	Pinaceae	1 Part
Ananta (sveta sariva)	Root	*Ichnocarpus Frutescens*	Apocynaceae	1 Part
Kirata (kiratatikta)	Plant	*Andrographis paniculata*	Acanthaceae	1 Part
Rasana (rasna)	Root	*Vanda roxburghii*	Orchidaceae	1 Part
Sinduvara (nirgundi)	Leaf	*Vitex negundo*	Verbenaceae	1 Part
Ugragandha (vaca)	Rhizome	*Acorus calamus*	Araceae	1 Part
Tarkari (agnimantha)	Root	*Premna serratifolia*	Verbenaceae	1 Part
Sigru	Stem & Bark	*Moringa oleifera*	Moringaceae	1 Part
Pippali	Fruit	*Piper longum*	Piperaceae	1 Part
Cavya	Stem	*Piper chaba*	Piperaceae	1 Part
Citraka	Root	*Plumbago zeylanica*	Plumbaginaceae	1 Part
Sunthi	Rhizome	*Zingiber mioga*	Zingiberaceae	1 Part
Ghunadayita (ativisa)	Root	*Aconitum heterophyllum*	Ranunculaceae	1 Part
Markava (bhrngaraja)	Flower	*Eclipta alba*	Asteraceae	1 Part
Pippali mula (Pippali)	Root	*Piper longum*	Piperaceae	1 Part

Chloroform and aqueous extract of *Ichnocarpus Frutescens* lowers the fasting blood sugar level in diabetic rats and increases the glucose tolerance.[11] *Andrographis paniculata is* used in acute upper respiratory tract infection.[12] However, a review studies reported few spontaneous adverse events of *A. paniculata*.[13] *Vanda roxburghii* another ingredient of *AKC* preparation can be topically used as wound healing potential in rats.[14] *Vitex negundo* has cyclooxygenase - 2 inflammatory cytokine mediated inflammation inhibitory activity.[15] *V. Negundo* has anti-tussive effect devoid of toxicity such as no signs of neural impairment and acute behavioral toxicity.[16] *Acorus calamus* has antispasmodic effect by calcium channel blocking activity.[17] *Moringa oleifera* has hepatoprotective activity[18] nephrotoxicity reducing activity.[19] *Piper chaba* has moderate diuretic activity only at the highest dose[20] and gastro-protective activity.[21] *Cedrus deodara* has anti-hyperlipidemic activity in animal model.[22]

AIMS AND OBJECTIVES

Patient in many regions have easy access to Ayurvedic medicine at a cheaper price depending on their choice. Ayurvedic medicine could be a potential alternative in the cases where expensive and extensive procedures of clinical investigations are needed. Considering the widespread use of Ayurveda as the popular form of traditional medicine in Bangladesh, one cannot emphasize enough the need for establishing the safety profiles of Ayurvedic drugs. Keeping in mind, study of *AKC* was carried out to explore its

wide spectrum toxicological aspects in animal model. We were also aimed to justify the pharmacological uses of AKC in some extent. The research was carried out in order to characterize the toxicological profile of the Ayurvedic medicinal preparation *AKC* on the following aspects: a) Serum protein/albumin b) Lipid profile c) Liver function test d) Kidney function test e) Serum Uric acid.

HYPOTHESIS

Previous research studies have been reported many activities of *AKC*. In our present study, we thought that *AKC* may have a wide range of potential activities. *AKC* may improve the lipid profile; including the lowering of LDL, VLDL, TG. It may helps to enhance the HDL levels in the blood. It is also thought that *AKC* may affect the serum protein/albumin, in the animal model.

MATERIALS AND METHODS

Animals and housing: Forty eight-week old albino healthy rats (*Rattus novergicus* : *Sprague Dawley* strain, weighed 50-70 g) of both sexes, bred and maintained at the Animal House of the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh were used in the experiment. The animals were housed in a well ventilated hygienic house under constant environmental and adequate nutritional conditions throughout the period of the experiment. All of the rats were kept in plastic cages having dimensions of 30 x 20 x 13 cm and soft wood shavings were employed as bedding in the cages. Feeding of animals was done ad libitum, along with drinking water and maintained at natural day night cycle.

All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals. Animals were handled in accordance with international principles guiding the use and handling of experimental animals (United States National Institutes for Health Publication, 1985). Experimental protocol was approved by Institutional Ethics Committee of the Department of Pharmacy, Jahangirnagar University (approval no. JU/DP/10/11).

Rats were randomly divided into 4 groups of 10 animals per sex. Ten rats were taken for each group for both control and drug group. The Liquid Ayurvedic formulation *AKC* was collected from Sri Kundeswari Aushadhalaya Ltd., Chittagong, Bangladesh. The liquid drug was administered per oral route at a dose of 40 ml/kg body weight for toxicological experiment. After 46-days treatment period, the animals were fasted for 18 hours after the last administration. The animals were anaesthetized using ketamine 500 mg per kg through intraperitoneal route. *Collection and preparation of plasma:* Blood samples were collected from post venacava and transferred into heparinised tubes immediately. Blood was then centrifuged at 4,000 g for 10 minutes using bench top centrifuge (MSE Minor, England) to remove red blood cells and recover plasma. Plasma samples were separated and were collected using dry Pasteur pipette and stored in the refrigerator for analyses. All analyses were completed within 24 hour of sample collection. *Biochemical Parameters:* Biochemical studies involved analysis of parameters such as total protein, serum albumin, blood urea nitrogen, bilirubin, creatinine. Total protein content of the samples was assayed by the Biuret method.[23] Serum albumin concentration

was determined using the method of Dumas et al., in 1997.[24] Triglycerides and total cholesterol concentration as well as protein content were evaluated using assay kits (purchased from Sigma Chemical Co, St Louis, MO, USA). Serum total cholesterol and high-density lipoprotein cholesterol were determined using Randox Laboratory kit reagents. Serum triglyceride level was estimated using Randox Laboratory test kit and VLDL-cholesterol was calculated using the formula TG/2.2 mmol/l. Low density lipoprotein cholesterol was determined by differential subtraction of the sum of the cholesterol fractions from the total cholesterol. The method of Evelyn and Malloy (1938) was employed to determine the serum bilirubin concentration of the samples.[25] The procedure of Tietz et al (1994) was used to determine serum creatinine concentration while the serum urea concentration was determined by the method of Kaplan (1965).[26, 27] The absorbance of all the tests were determined using spectrophotometer (Model No. UV – 1601 PC)

STATISTICAL ANALYSIS

The group data are expressed as Mean ± SEM (Standard Error of the Mean). Unpaired "t" tests were conducted for statistical significance tests. SPSS (Version 16) was used for data analysis. Differences between groups were considered significant at $p < 0.05$.

RESULTS

In this experiment the total protein and albumin content in the plasma, lipid profiles, liver function test, kidney function test and serum uric acid level were determined. The results of the toxicological studies are in Table 2.

Total protein and albumin content in the plasma were significantly ($p<0.05$) increased in the *AKC* group (Table 2) their corresponding control group. Triglyceride content in the plasma was significantly ($p<0.05$) decreased in the *AKC* group than the corresponding control group. On the contrary, total cholesterol, LDL, VLDL and HDL content in the plasma were remaining unchanged ($p>0.05$). Bilirubin contents were decreased significantly ($p<0.05$) in male *AKC* group than the corresponding control group. Creatinine contents in the plasma was raised significantly ($p<0.05$) in the *AKC* group. Urea levels in the blood were significantly decreased ($p<0.05$) in the *AKC* group than their corresponding controls.

Table 2: Biochemical parameters of Control and *Arkadi Kvatha Curna*
[Values presented in mean (mg/dl) ± standard error mean (SEM)]

Parameters	Male Rats			Female Rats		
	Control (n=10)	***AKC* (n=10)**	**p**	**Control (n=10)**	***AKC* (n=10)**	**p**
Total protein	5254.19 ± 81.68	6129.45±81.39	0.001***	5870.13±141.52	6674.49±110.47	0.001***
Albumin	4178.42±101.02	5321.25±86.35	0.001***	4605.23 ± 82.41	6198.20 ±94.56	0.001***
Triglycerides	102.91 ± 1.73	56.65 ± 1.58	0.001***	94.16 ± 3.06	48.86 ± 1.89	0.001***
Total Cholesterol	74.68 ± 1.69	73.89 ± 2.07	0.439	72.04 ± 1.54	74.07 ± 1.32	0.304
VLDL	16.63 ± 0.67	15.70 ± 0.53	0.388	16.91 ± 0.52	16.45 ± 0.54	0.793
LDL	19.91 ± 0.75	21.02 ± 0.62	0.399	18.32 ± 0.62	20.22 ± 0.71	0.068
HDL	34.25 ± 0.91	34.12 ± 0.79	0.901	31.73 ± 0.91	32.23 ± 0.89	0.581
Bilirubin	0.12 ± 0.003	0.07 ± 0.002	0.001***	0.09 ± 0.004	0.13 ± 0.01	0. 001
Creatinine	1.08 ± 0.026	1.10 ± 0.033	0.091***	0.88 ± 0.031	1.66 ± 0.035	0.001***
Urea	73.87 ± 1.18	58.06 ± 1.06	0.001***	54.90 ± 0.98	45.18 ± 0.83	0.001***
Uric acid	2.43 ± 0.06	2.49 ± 0.05	0.041	3.02 ± 0.09	3.36 ± 0.05	0.024

Note: *p<0.05, **p<0.01, ***p<0.001

DISCUSSION

The present study was conducted to evaluate the effect of traditional Ayurvedic preparation *Arkadi Kvatha Curna* (*AKC*) on various biochemical parameters of the animal's plasma after chronic administration.

Increased level of total protein and albumin content were found in the *AKC* group. The phytochemical constituents of AKC are responsible for this raised level of total protein and albumin. Triglycerides level in the plasma was decreased after the administration of *AKC*. The component of *AKC* such as *Cedrus deodara*, *Calotropis gigantea* and *Ichnocarpus frutescens* might be responsible for lowering the triglyceride levels in the plasma. Previous research findings are in favor with our present result. The component of AKC such as *Calotropis gigantea* can reduce triglyceride level in animal model.[22] Calotropis contains polysterols and di-(2-ethylhexyl) phthalate which restore normal triglyceride level[28] and show beneficial activity in hyperlipidemia.[29] *Ichnocarpus frutescens* reduces triglyceride in high-fat diet animal model.[30] Furthermore, *Andrographis paniculata* reduces triglyceride level in high fructose fat fed rats.[31] Most active compound of *Andrographis paniculata* is andrographolide which has an important role in hypolipidemic effects. A decreased level of bilirubin content in the plasma was found in the *AKC*. Bilirubin lowering activity could be beneficial for the liver disorder patient. Higher level of Creatinine was found in the *AKC* group. Higher level of creatinine may result from the decreased synthesis or increased functional capacity of tubular excretion.[32] A decreased level of urea was also found in the *AKC* group. Probably di-(2-ethylhexyl) phthalate of *Calotropis gigantea* is responsible for lowering blood urea. Habib et al., in 2012 reported that di-(2-ethylhexyl) phthalate present in *Calotropis gigantea* which can restore the normal level of blood urea level.[28] Increased level of creatinine and decreased level of urea indicates that this preparation improve kidney function. So, it can be used in nephropathy.

CONCLUSION

Chronic administration of *Arkadi Kvatha Curna* in animal model leads to various biochemical results. Ayurvedic preparation generally consists of multiple plants and their parts. Multiple plant parts contain more than one chemical constituent in a single Ayurvedic formulation like *AKC* that possesses a wide range of pharmacological activities. It is hard to stay in one pharmacological uses. However, present study shows that *Arkadi Kvatha Curna* preparation may contribute in hyper-triglyceridemia and nephropathy.

REFERENCES

1. Cragg GM, Newman DJ. Natural products drug discovery in the next millennium. 2001; 39 Suppl 1:8-17. Epub 2001/01/01

2. Chopra A, Doiphode VV. Ayurvedic medicine; Core concept; therapeutic principles and current relevance. The Medical clinics of North America; 2002; 86 (1): 75-89, vii. Epub 2002/01/25

3. Gogtay N. J. et al. The use and safety of non-allopathic Indian medicines; Drug safety: an international journal of medical toxicology and drug experience. 2002; 25 (14):1005-19. Epub 2002/11/01

4. Thatte UM et al; The flip side of Ayurveda, Journal of postgraduate

medicine, 1993; 39 (4): 179-82, 82a-82b. Epub 1993/10/01
5. Chopra A et al; Efficacy and safety of Ayurvedic medicines: Recommending equivalence trial design and proposing safety index 2010 July 1, 2010. 175-80
6. Anonymous. Bangladesh National Formulary of Ayurvedic Medicine 1992
7. Argal A, Pathak AK. CNS activity of Calotropis gigantea roots, Journal of ethnopharmacology. 2006; 106 (1):142-5
8. Adak M, Gupta JK; Evaluation of anti-inflammatory activity of Calotropis gigantean in various biological systems. Nepal Medical College journal: NMCJ. 2006; 8 (3):156-61. Epub 2007/01/06
9. Yimam M, Brownell L, Hodges M, Jia Q. Analgesic Effects of a Standardized Bioflavonoid Composition from Scutellaria baicalensis and Acacia catechu. Journal of Dietary Supplements. 2012; 9 (3):155-65
10. Malathy NS, Sini S. Antimicrobial activities of Ichnocarpus frutescens (L.) R.Br. and Hemidesmus indicus R.Br. Roots. Ancient science of life. 2009; 28 (4):13-5. Epub 2009/04/01
11.Poolsup N, Suthisisang C, Prathanturarug S, Asawamekin A, Chanchareon U. Andrographis paniculata in the symptomatic treatment of uncomplicated upper respiratory tract infection: systematic review of randomized controlled trials. Journal of clinical pharmacy and therapeutics; 2004; 29(1):37-45. Epub 2004/01/30
12. Coon JT, Ernst E. Andrographis paniculata in the treatment of upper respiratory tract infections: a systematic review of safety and efficacy. Planta medica. 2004; 70 (4): 293-8.
13. Puri A ey al; Immunostimulant agents from Andrographis paniculata. Journal of natural products; 1993; 56 (7): 995-9
14. Chattopadhyay et al. Vitex negundo inhibits cyclooxygenase-2 inflammatory cytokine-mediated inflammation on carragenan-induced rat hind paw edema. Pharmacognosy research; 2012; 4(3):134-7
15. Haq RU, Shah AU, Khan AU, Ullah Z, Khan HU, Khan RA, et al. Antitussive and toxicological evaluation of Vitex negundo. Natural product research; 2012; 26 (5): 484-8. Epub 2011/08/04
16. Gilani AU et al; Antispasmodic effect of Acorus calamus Linn is mediated through calcium channel blockade. Phytotherapy research: 2006; 20 (12):1080-4. Epub 2006/09/30
17. Muthuraman A, Singh N. Attenuating effect of hydroalcoholic extract of Acorus calamus in vincristine-induced painful neuropathy in rats. Journal of natural medicines; 2011; 65 (3-4): 480-7
18. Rajendran R, Krishnakumar E. Anti-Arthritic Activity of Premna serratifolia Linn., Wood against Adjuvant Induced Arthritis. Avicenna journal of medical biotechnology; 2010; 2(2):101-6
19. Selvam TN et al; Antioxidant and tumor cell suppression potential of premna serratifolia linn leaf. Toxicology international; 2012; 19 (1): 31-4
20. Morikawa T et al; New amides and gastroprotective constituents from the fruit of Piper chaba; Planta medica. 2004; 70(2):152-9. Epub 2004/03/03.
21. Muruganandan S et al; Anti-inflammatory activity of Syzygium cumini bark; Fitoterapia. 2001; 72 (4): 369-75
22. Patil S et al; Antihyperlipidemic potential of Cedrus deodara extracts in monosodium glutamate induced obesity in

neonatal rats. Indian journal of pharmacology; 2011; 43 (6): 644-7
23. Wright PJ, Plummer DT; The use of urinary enzyme measurements to detect renal damage caused by nephrotoxic compounds; Biochemical pharmacology. 1974; 23 (1): 65-73. Epub 1974/01/01
24. Dumas BT, Watson WA, Biggs HG; Albumin standards and the measurement of serum albumin with bromcresol green. 1971. Clinica chimica acta; international journal of clinical chemistry. 1997; 258 (1): 21-30. Epub 1997/02/03
25. Evelyn KA, Malloy HT; Microdetermination of oxyhemoglobin, methemoglobin and sulfhemoglobin in single sample of blood; Journal of Biological Chem; 1938; 126 (2): 655-62.
26. Tietz N, Pruden E, O S-A. Tietz textbook of Clinical Chemistry; eds. BCaAE, editor: WB Saunders Company London; 1994.
27. Kaplan M. Standard Method of Clinical Chemistry. Meites, editor: Academic Press Inc., New York; 1965
28. Habib MR, Karim MR. Antitumour evaluation of di-(2-ethylhexyl) phthalate (DEHP) isolated from Calotropis gigantea L. flower / Evaluacija antitumorskog djelovanja di-(2-etilheksil)-ftalata (DEHP) izoliranog iz cvjetova Calotropis gigantea L. Acta pharmaceutica (Zagreb, Croatia). 2012; 62 (4): 607-15. Epub 2013/01/22
29. Jenkins DJ, Kendall CW. Plant sterols, health claims and strategies to reduce cardiovascular disease risk; Journal of the American College of Nutrition; 1999; 18 (6): 559-62. Epub 1999/12/29
30. Saravanan M et al; Antihyperlipidemic activity of Ichnocarpus frutescens in triton WR-1339-induced and high-fat diet animals. Pharmaceutical biology; 2011; 49 (10):1074-81. Epub 2011/05/20
31. Nugroho AE, Andrie M, Warditiani NK, Siswanto E, Pramono S, Lukitaningsih E. Antidiabetic and antihiperlipidemic effect of Andrographis paniculata (Burm. f.) Nees and andrographolide in high-fructose-fat-fed rats; Indian journal of pharmacology; 2012; 44 (3): 377-81. Epub 2012/06/16
32. Traynor J, Mactier R, Geddes CC, Fox JG. How to measure renal function in clinical practice. BMJ; 2006; 333 (7571): 733-7

CORRESPONDING AUTHOR
Md. Mamun Al-Amin
Department of Pharmacy
North South University
Plot–15, Bashundhara
Dhaka – 1229, Bangladesh
Tel: +880-1927077102; Fax: 8852016
e-mail: bd_pharmacy@yahoo.com

Source of support: Nil
Conflict of interest: None Declared