

## Studies of Lipid Profile, Liver and Kidney Function Parameters of Female Rat Plasma After the Administration of “Khadiraristha”

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In this study, Khadiraristha (KDR), a traditional Ayurvedic preparation used in dermatological disorder has been investigated for its biochemical effects and in some extent justify the pharmacological uses under the stated circumstances. Khadiraristha was administered orally to female albino rat for 46 days. After the treatment period animals were fasted for 18 hours. Biochemical parameters including: Total protein, Serum albumin, Blood Urea Nitrogen, Bilirubin, Liver enzymes (sGPT, sGOT, ALT) were determined in the plasma. KDR significantly increased ( $p<0.05$ ) Total protein, Serum Albumin, Total cholesterol, HDL, Liver enzymes (sGPT, sGOT, ALT) and Urea levels than their corresponding controls while decreased ( $p<0.001$ ) Triglyceride and Creatinine levels. Hence, KDR may cause liver toxicity (due to the increased liver enzymes) and kidney dysfunction (due to the increased level of Creatinine). Our results suggest that KDR might have a noble role in those patients who have hypertriglyceridemia and hypercholesterolemia as it improves Triglyceride and HDL levels.

**Key words:** Khadiraristha (KDR), Biochemical study, Pharmacology

### Khadiraristha Uygulaması Sonrasında Dişi Sıçan Plazmasında Lipid Profili, Karaciğer ve Böbrek Fonksiyonları Parametreleri Üzerinde Çalışmalar

Bu çalışmada, geleneksel ayurvedik bir preparat olan Khadiraristha'nın (KDR) dermatolojik bozukluklardaki kullanımı, biyokimyasal etkileri ve bazı durumlarda belirtilen koşullar altındaki farmakolojik kullanımları araştırılmıştır. KDR, dişi albino sıçanlara 46 gün oral yolla uygulanmıştır. Uygulama periyodundan sonar hayvanlar 18 saat aç bırakılmışlardır. Sonrasında plazmada, total protein, serum albumin, kan üre azot, bilirubin ve karaciğer enzimleri (sGPT, sGOT, ALT) tayinleri yapılmıştır. KDR uygulaması total protein, serum albumin, total kolesterol, HDL, karaciğer enzimleri (sGPT, sGOT, ALT), ve üreyi kontrollere göre istatistiksel olarak anlamlı bir seviyede arttırmış ( $p<0.05$ ) buna karşılık trigliserid ve kreatinin seviyelerini ise istatistiksel olarak anlamlı bir seviyede azaltmıştır ( $p<0.001$ ). Bu sonuçlara göre KDR karaciğer toksisitesine (artan karaciğer enzimleri nedeniyle) ve böbreklerde fonksiyon bozukluğuna (kreatinine seviyesindeki düşme nedeniyle) sebep olduğu düşünülebilir. Bizim sonuçlarımız, KDR'nin trigliserid ve HDL seviyelerini düzeltebilmesi nedeniyle hipertrigliseridemi ve hiperkolesterolemili hastalarda faydalı bir rolü olabileceğini göstermektedir.

**Anahtar kelimeler:** Khadiraristha (KDR), Biyokimyasal çalışmalar, Farmakoloji

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## INTRODUCTION

A large numbers of modern medicinal agents have been sourcing from the nature (1). Natural plants and their constituents are giving the primary ideas about the treatment of new disease. Hence, natural medicine like Ayurvedic drugs are still remains a popular

ensured scientifically (4,5). Moreover, the conflict between traditional medicines and allopathic medicine are needed to be addressed scientifically both in vivo and in vitro experimental model. Ayurvedic preparation such as *Khadiraristha* is a popular medicine used in dermatological disorders, glandular & splenic enlargement, swelling,

**Table 1.** The plants and ingredients used in the formulation of *Khadiraristha* (KDR) (8-11).

Name of Plants	Used parts	Botanical Name	Family	Amount
<i>Khadira</i>	Heart-wood	<i>Acacia catechu</i>	Leguminosae	2.40 kg
<i>Devadaru</i>	Heart-wood	<i>Cedrus deodara</i>	Pinaceae	576 g
<i>Bakuci</i>	Seed	<i>Psoralea corylifolia</i>	Leguminosae	960 g
<i>Darvi (daru haridra)</i>	Stem	<i>Cosciniun fenestratum</i>	Menispermaceae	960 g
<i>Haritaki</i>	Fruit powder	<i>Terminalia chebula</i>	Combretaceae	960 g
<i>Bibhitaka</i>	Fruit powder	<i>Terminalia bellirica</i>	Combretaceae	960 g
<i>Amalaki</i>	Fruit powder	<i>Embelica Officinalis</i>	Euphorbiaceae	98.30 L
Water for Decoction reduced to				12.28 L 2.40 kg
<i>Dhataki</i>	Flower	<i>Woodfordia fruticosa</i>	Lytheraceae	960 g
<i>Kankola</i>	Fruit	<i>Piper cubeba</i>	Piperaceae	48 g
<i>Nagakesara</i>	Flower	<i>Mesua ferrea</i>	Clusiaceae	48 g
<i>Jatiphala</i>	Seed	<i>Myristica fragrans</i>	Myristicaceae	48 g
<i>Lavanga</i>	Flower	<i>Eugenia caryophyllus</i>	Myrtaceae	48 g
<i>Ela</i>	Seed	<i>Elettaria cardamomum</i>	Zingiberaceae	48 g
<i>Tvak</i>	Stem & Bark	<i>Cinnamomum zeylanicum</i>	Lauraceae	48 g
<i>Krsna (pippali)</i>	Fruit	<i>Piper longum</i>	Piperaceae	48 g

practice in the subcontinent including India, Sri Lanka and other countries like Bangladesh (2,3). Ayurvedic preparations have a wide access to the large number of population in these countries. The acceptances of these medicines are 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 Ayurvedic medicines are not

tumor & anemia (6). *Khadiraristha* is a preparation of heart wood *Acacia catechu* (*Khadira*) with some other medicinal plants in small amount. *Khadiraristha* is included in the Bangladesh National Formulary of Ayurvedic Medicine 1992 (7). Bangladesh National Formulary of Ayurvedic Medicine is compiled by the National Unani and Ayurvedic Formulary Committee and published by the Bangladesh 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. At present a large number of Ayurvedic manufacturers are manufacturing and marketing the Classical Ayurvedic Medicinal Preparation.

The usual dose of *Khadiraristha* is 12 to 24 ml. It is used for many therapeutic purpose including a dermatological disorders, glandular enlargement, localised abdominal swelling or tumour, intestinal parasites / worms infestation, splenic enlargement, tumour and anaemia (12). Composition of KDR is shown in table 1. The principle component of KDR is *Acacia catechu* (*Khadira*) which has an effect on cell mediated and humoral immunity (13), hypotensive (14) and analgesic action (15), anti-inflammatory action (15). *Cedrus deodara* has shown its antihyperlipidemic activity in animal model (16) and anti-inflammatory and analgesic activity (17). Embryotoxicity (18) and estrogenic activity in yeast transactivation assay (19) are shown by *Psoralea corylifolia*. *Terminalia bellirica* was not proven any oral toxic effect (20) and found to prevent the hepatotoxicity (21). *Emblica officinalis* possesses antioxidant (22), gastroprotective (23), cytoprotective, immunomodulating (24) and radiation protective activity (22).

Reduction of SGOT, SGPT, glutathione-S-transferase levels and DNA synthesis (25) and selective cytotoxicity against tumor and non-tumor cell lines (26) were shown by *Embelica Officinalis*. Interestingly, many papers have reported that the hepato-protective effect of *Woodfordia fruticosa* as it reduces the elevated levels of serum ALT, AST, ALP and BUN (Blood Urea Nitrogen) (27-29). *Elettaria cardamomum* traditionally used to treat skin disease and digestion problem (30). *E. cardamomum* can reduce blood pressure, enhances fibrinolysis and improves antioxidant status (31). The methanol extract of *Cinnamomum zeylanicum* stem bark has antihypertensive effects due to its ability to enhance the endogenous NO production and regulation of dyslipidemia (32).

#### *Aim of the study*

Ayurvedic medicine could be a potential alternative in the cases where expensive and extensive procedures of clinical investigations are needed. Patient in many regions have wide access to Ayurvedic medicine at a cheaper price depending on their choice. Considering the widespread use of *Khadiraristha* (KDR) as the popular form of traditional medicine in Bangladesh, one cannot emphasize enough the pharmacological uses of this drug. Keeping in mind, the present research work was carried out to investigate the biochemical profiles of animals those were given KDR preparation.

#### *Hypothesis*

In our present study we thought that *Khadiraristha* (KDR) may influence on liver, kidney functions and affect lipid profiles. KDR may improve the cholesterol profile; including the lowering of LDL, VLDL, TG. It may helps to enhance the HDL levels in the blood. It is also thought that *KDR* can improve the liver enzymes and may affect the serum protein/albumin, serum enzymes levels in the body.

## **MATERIALS AND METHODS**

The liquid ayurvedic formulation *Khadiraristha* (KDR) was collected from Sri Kundeswari Aushadhalaya Ltd., Chittagong, Bangladesh. The liquid KDR preparation was administered per oral route at a dose of 0.20 ml/kg body weight to maintain optimal dosage accuracy without contributing much to the total increase in the body fluid. Forty eight-week old albino female rats (*Rattus norvegicus*: Sprague-Dawley strain), bred and maintained at the Animal House of the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh were used in the experiment. These animals were apparently healthy and weighed 50-70 g. The animals were housed in a well ventilated hygienic experimental animal house under constant environmental and adequate nutritional conditions throughout the period of the experiment. All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals (United States National Institutes for

Health Publication, 1985). Experimental protocol was approved by Institutional Ethics Committee of the, Faculty of Biological Science, Department of Pharmacy, Jahangirnagar University.

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. They were fed with "mouse chow" (prepared according to the formula developed at BCSIR, Dhaka). All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals. Before starting an experiment the animals were carefully marked on different parts of their body, which was later used as identification mark for a particular animal, so that the response of a particular rat prior to and after the administration could be noted separately. A group of equal number of rat as the drug treated group was simultaneously employed in the experiment. They were administered with distilled water as placebo as par the same volume as the drug treated group for the same number of days and this group served as the control. Prior to the experiment, they were randomly divided into 2 groups of 10 animals. Thus ten rats were taken for each group for both control and the experimental group. After acclimatization, administration of the ayurvedic medicinal preparation was done by intra-gastric syringe. At the due of the 46-days treatment period, the animals were fasted for 18 hours and also twenty-four hours after the last administration, the animals were anaesthetized using i.p. Ketamine (500 mg/kg i.p.). Blood samples were collected from post vena cava and transferred into heparinised tubes immediately.

#### *Preparation of plasma*

Blood was then centrifuged at 4,000 g for 10 min 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 h of sample collection.

#### *Determination of Biochemical Parameters*

Biochemical studies involved analysis of parameters such as total protein, serum albumin, blood urea nitrogen, bilirubin (total and direct), creatinine, and liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). Total protein content of the samples was assayed by the Biuret method (35). Serum albumin concentration was determined using the method of Dumas BT (1997) (36). 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 triacylglycerol level was estimated using Randox Laboratory test kit and VLDL-cholesterol was calculated using the formula  $TG/2.2$  mmol/l. Lowdensity lipoprotein (LDL) 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 (37). 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) (38,39). Alkaline phosphatase activities were determined using the method as described by King and King (1954) (40). The absorbances of all the tests were determined using spectrophotometer (UV-Visible Spectrophotometer Model No. UV-1601 PC).

#### *Statistical analysis*

The group data are expressed as Mean  $\pm$  SEM (Standard Error of the Mean). Unpaired "t" tests were conducted for statistical significance tests. SPSS (Statistical Package for Social Science) for WINDOWS (Version 11) was applied for data analysis. Differences between groups were considered significant at  $p < 0.05$ , 0.01 and 0.001.

## RESULTS

Total protein and albumin content in the plasma were significantly ( $p=0.001$ ) increased in the *Khadiraristha* (KDR) group than their corresponding control group (see Table 2). Although triglyceride level was significantly ( $p=0.001$ ) decreased but total cholesterol level was significantly ( $p=0.008$ ) raised in the KDR group compared to their control group. HDL contents in the plasma was significantly ( $p=0.047$ ) increased in the KDR rats compared to the control group. Moreover, LDL and VLDL content in the plasma were unchanged. Liver function testing parameters such as, Bilirubin content in the plasma was unchanged. All of the liver enzymes (sGPT, sGOT and ALP) were significantly ( $p=0.001$ ) elevated in the KDR rats compared to their control group. Creatinine content in the plasma was significantly ( $p=0.001$ ) decreased in the KDR group compared to their control group. Urea content in the plasma was significantly ( $p=0.001$ ) increased in the KDR rats than their corresponding control group.

## DISCUSSION

The present study was conducted to evaluate the effect of traditional Ayurvedic preparation *Khadiraristha* (KDR) on various biochemical parameters in the animal's plasma including a) Serum protein/albumin b) Lipid profile c) Liver function test d) Kidney function test and e) Serum enzymatic activity.

One of the main findings of this study is, KDR reduced Triglycerides level and improves HDL level in the plasma. The phytochemical constituents of KDR such as polysterols (found in *Cedrus deodara*) might be responsible for lowering the triglyceride contents in the plasma (16). Moreover, the components of KDR such as; *Acacia Catechu*, *Cedrus deodara*, *Cinnamomum zeylanicum* might also be accountable for triglyceride lowering and HDL improving activity as shown in previous studies (16,41). L-arginine analogue inhibits NO production in the blood and causes arterial hypertension, dyslipidemia and histological damages. In this case, the bioactive phytomolecules of *Cinnamomum*

**Table 2.** Biochemical parameters of *Khadiraristha* (KDR) and control female rats.

	Control (n=10)	KDR (n=10)	P
Total protein	5384.66±60.43	6315.27±50.95	0.001***
Albumin	4221.30±75.56	5716.27±101.33	0.001***
Triglycerides	97.96 ± 3.59	64.93 ± 1.28	0.001***
Total Cholesterol	75.24 ± 1.64	80.96 ± .38	0.008**
VLDL	17.74 ± 0.43	18.62 ± .70	0.308
LDL	19.65 ± 0.69	21.81 ± 1.21	0.143
HDL	34.37 ± 1.01	37.67 ± 1.15	0.047*
Bilirubin	0.07 ± 0.004	0.073 ± 0.001	0.805
Creatinine	0.97 ± 0.04	0.72 ± 0.017	0.001***
Urea	57.53 ± 1.24	70.22 ± 1.82	0.001***
Uric acid	2.79 ± 0.09	2.96 ± 0.035	0.121
sGPT	50.16 ± 0.14	52.18 ± .14	0.001***
sGOT	82.50 ± 0.20	89.37 ± .19	0.001***
ALP	35.45 ± 0.10	38.28 ± 0.01	0.001***

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

*zeylanicum* (methanol extract) such as flavanoids and saponins possess antihyperlipidemic effects through its ability to increase the production of the endogenous NO (Nitric Oxide) (41).

Another important part of this study is the determination of kidney function parameters such as creatinine and urea. KDR reduced creatinine level and increased urea level which indicates poor kidney function. Poor kidney function may result due to the decreased functional capacity of tubular secretion (42).

KDR did not affect bilirubin production, but it increased liver enzymes (sGPT, sGOT and ALP) which reflect the liver injury. This finding is consistent with the previous finding (27,29). Increased level of liver enzymes may result by the phytochemical constituents of KDR such as *Embelica Officinalis* and *Coscinium fenestratum* (33). *Embelica Officinalis* may cause GSH (g-glutamyl cysteinyl glycine) depletion which is a non-protein thiol present in liver that plays a major role in the detoxification processes (25). Reduced level of GSH is unable to protect the oxidative action of the free radicals in the plasma. *Coscinium fenestratum* also induce hepatotoxicity by increasing serum AST, ALT, ALP and GGT concentration (34).

Finally, KDR increased total protein and albumin content due to the phytochemical constituents of *Coscinium fenestratum* which may enhance the total protein level in the plasma possibly due to the increase in globulin fractions and other serum proteins (43).

So, present study may suggest that the preparation of KDR can be recommended in hypertriglyceridemia, hypercholesterolemia patient to improve TG and HDL levels in the blood.

## CONCLUSION

Ayurvedic preparation generally consists of multiple plants and their different parts. Multiple plant parts contain more than one chemical constituent in a single Ayurvedic preparation like *Khadiraristha* that possess a wide range of pharmacological and toxicological activities. It is hard to stay in

one pharmacological uses and needed to determine the biochemical profile after the oral administration at usual dose. Except the Liver and kidney dysfunction, Triglyceride lowering and HDL elevating activities are particularly beneficial. Further molecular level studies should be conducted to explore the chemical/(s) responsible to exhibit the beneficial pharmacological and toxicological profiles of KDR preparation.

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