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Research Article

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Toxicological studies of Karpura Rasa on the basis of lipid profile, liver function and kidney function in rat plasma after chronic administration

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ABSTRACT

Karpura Rasa (KRP), a classical Ayurvedic preparation which is used in diarrhea and dysentery, was studied for its toxicological aspects after chronic administrations into the biological system. Rat of both Sexes were used as the experimental model. In male, as well as female group there were statistically very highly significant increases in both the total protein and albumin content in the plasma. Statistically very highly significant decrease in the Triglycerides (TG) and total cholesterol content were observed in both sexes of experimental animal. Considerable decrease in the low density lipoprotein (LDL) and high density lipoprotein (HDL) were noted, although decrease in very low density lipoprotein (VLDL) was not statistically significant. Irrespective of sexes, statistically highly significant decrease in the bilirubin content, creatinine and urea in the plasma were observed in the experimental animal. There was an increase in the uric acid content in the plasma of male rats but this was not significant. Similar result was also observed in female rats. Serum glutamic pyruvic transaminase (sGPT) and serum glutamic oxaloacetic transaminase (sGOT) activities in the plasma were decreased to a very highly significant level in both of the experimental groups. Besides, a negligible change in the alkaline phosphatase (ALP) activity in the plasma was noted which was statistically insignificant.

Key words: Karpura Rasa, Ayurvedic, Lipid Profile, Liver function, Kidney function.

INTRODUCTION

For treatment of various types of chronic ailments Ayurvedic metallic preparations with herbal liquids are being used and widely recommended in the Indian subcontinent since the seventh century BC. Karpura Rasa is included (page 184) 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]. “Karpura Rasa” (KRP) is one of unique metallic herbal Ayurvedic preparation, used mainly in chronic diarrhea, dysentery and fever. In this preparation, metallic compound hingula (HgS) is used with other medicinal plans. This metal is found to be chelated with organic ligands derived from these plants liquids. Artificially prepared hingula after purification taken along with herbal liquids. Thus, this makes these elements easily assimilable, eliminating their harmful effects and enhancing their biocompatibility [1]. Moreover, hingula itself useful in the treatment of rheumatism, fever and chronic rhinitis [2].

Different parts of five medicinal plants are also used in KPR, where plants are themselves renowned for their medicinal and therapeutic uses. The anti-fungal, antibacterial and antioxidant potentials of essential oil and acetone extract of Jatiphala *(Myristica fragrans)* were reported by Singh *et al.* (2004). Olaleye *et al.* (2006) confirmed the presence of alkaloids, saponins, anthraquinones, cardiac glycosides, flavonoids and phlobatanins in *Myristica fragrans*. Ahiphena *(Papaver somniferum)* is well known for its wide ranged uses in the treatment of epilepsy,

diarrhea, sperm problems, Insomnia [5]. Uddin *et al.* (2006) reported Mustaka *(Cyperus rotundus)* to be very useful in the treatment of fevers, digestive system disorders, dysmenorrheal and other diseases. For the treatment of amoebiasis, chronic bronchitis, diabetes and locally for boils and ulcers, Indrayava *(Wrightia antidysenterica)* is being used from ancient time [7]. Karpura *(Cinnamomum camphora)* is known for its use for modulating sexual activity, contraception, inducing abortion, and reducing milk production in lactating women [8]. Camphor is used for different purposes such as stimulation of circulatory and respiratory systems, psychological stimulation, and cosmetics (as sun protection) for external use [9-10]. Recently, investigations have shown that camphor containing compounds have uterotrophic [11], antitussive [12] anticonvulsant [13], nicotinic receptor blocking [14], antiimplantaion [15], antiestrogenic as well as estrogenic [16], activities, and reduced serum triglyceride and thyroid hormone [17]. In Iran's folk medicine, camphor has been used both as an aphrodisiac and antiaphrodisiac.

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, the present scenario this research work on Ayurvedic formulation, Karpura Rasa (KRP) explores a spectrum of its toxicological aspects utilizing experimental animals. The objective is to have a better understanding of the possible toxicological profile of the drug under study and, to some degree, to decide how justifiable the use of this drug is under the stated circumstances. The project will eventually result in supplementing and complementing the existing health care facilities and, in the long run, will ensure total coverage of the population in terms of public health.

EXPERIMENTAL SECTION

Drugs, Chemicals and Reagents

For the toxicological study Karpura Rasa (KRP) was collected from Sree Durga Aushadhalaya Ltd, Chittagong. All other reagents and chemicals that were used in this work were purchased from Sigma Chemical Company and were prepared in all glass-distilled water.

Table 1: Ingredients of Karpura Rasa (KRP)

Ayurvedic/ Traditional Name	Parts Used	Name/ Botanical Name	Family	Amount used
Hingula	Purified HgS	Cinnabar	----	1 part
Ahiphena	Seed	*Papaver somniferum*	Papaveracea	1 part
Mustaka	Rhizome	*Cyperus rotundus*	Cyperaceae	1 part
Indrayava	Seed	*Wrightia antidysenterica*	Apocynaceae	1 part
Jatiphala	Seed	*Myristica fragrans*	Myristicaceae	1 part
Karpura	Wood	*Cinnamomum camphora*	Lauraceae	1 part

Experimental Animals

For the purpose of toxicological experiment forty eight-week aged Albino rats (*Rattus novergicus*. Sprague-Dawley strain,) of both sexes, bred and maintained at the Animal House of the Department of Pharmacy, Jahangirnagar University . These animals were apparently healthy and weighed 50-70 gm. Throughout the entire period of experiment 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 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.

Experimental Design

Forty healthy rats of both sexes were used for the experiment. The rats were divided into four groups having ten animals in each group where two were male groups and other two were female groups. For both of the sexes, one group was treated with KPR and another was used as a control. The control animals were administered with distilled water as placebo as par the same volume as the drug treated group for the same number of days. 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. For all the pharmacological studies the drugs were administered per oral route at a dose of 100mg/kg body weight. After acclimatization, administration of KPR was done by intra-gastric syringe between the hours of 10 am and noon. At the duration of the 90-day 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 01.0 Ketamine (500 mg/kg i.p.).

Blood Samples Collection and preparation of Plasma

Blood samples were collected from post vena cava and transferred into heparinised tubes immediately. 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 hour of sample collection.

Determination of Biochemical Parameters

Biochemical analysis was carried out on plasma, to assess the state of the liver and kidney. Biochemical studies involved analysis of parameters such as total protein, serum albumin, blood urea nitrogen (BUN), bilirubin (total and direct), creatinine, and liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). For lipid profile study, trirglycerides (TG), total cholesterol (TC) and high density lipoprotein (HDL) were determined but low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated. Total protein content of the samples was assayed by the Biuret method [18] where serum albumin concentration was determined using the method of Doumas *et al.* (1971). Triglycerides (TG) and total cholesterol (TC) 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. Low density 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. 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). Serum glutamic pyruvic transaminase (sGPT), serum glutamic oxaloacetic transaminase (sGOT) and alkaline phosphatase activities were determined using the method as described by King and King (1954).

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 ± SEM (Standard Error of the Mean). Unpaired "t" tests were done for statistical significance tests. SPSS (Statistical Package for Social Science) for WINDOWS (Ver. 11) was applied for the analysis of data. Differences between groups were considered significant at $p < 0.05$, 0.01 and 0.001.

RESULTS AND DISCUSSION

Lipid Profile

Statistically very high significant decrease in triglyceride (TG) (male. p = 0.001, female. p = 0.001) was found in the plasma of both male and female rats. Decrease in total cholesterol (TC) (male. p = 0.001, female. p = 0.001) of male and female was also very highly significant. Lowering of high density lipoprotein (HDL) was significant in male, this result is very highly significant (p = 0.001) in case of female rats. Decrease in low density lipoprotein (LDL) is also significant in the rats of both of sexes. On the contrary, the change in very low density lipoprotein (VLDL) was not significant. Like other plants constituents reduced TG level and it could be suggested that KPR increased lipase activity which hydrolyzed TG[23]. Among the lipids, increased blood level of TC, LDL and VLDL as well as lowered level of HDL has been identified as contributors in the development of hyperlipidemia [24], which is the consequences of, in majority of the cases, diabetes mellitus [25-27]. The elevation of lipid components is a risk factor for coronary heart disease [28]. KPR may act as inhibitor for enzyme such as hydroxyl-methyl-glutaryl-CoA reductase, which is the key enzyme in de novo cholesterol biosynthesis as has been suggested for some plants earlier [29-30]. This reduction could be beneficial in improving lipid metabolism and complications in diabetes [31] (Table 2).

Liver Function

In the male as well as female rats there was statistically very highly significant increase in the total protein (male. p = 0.001, female. p = 0.001) and the albumin (male. p = 0.001, female. p = 0.001) content of the plasma. These proteins are important liver function marker. Bilirubin, another liver function indicator, was found to be decreased by KPR to very significant level in both of the sexes. According to Naganna (1989), increase in bilirubin is indicating the abnormal liver function which may be the results of higher synthetic function of the liver. Result is indicating the normal liver function which is contradictory with the total protein and albumin observation. Serum glutamic pyruvic transaminase (sGPT) content in the plasma, irrespective of sexes, were decreased to very highly significant level (male. p = 0.001, female. p = 0.001). Similar results (male. p = 0.001, female. p = 0.001) were also observed in case of serum glutamic oxaloacetic transaminase (sGOT). These results also indicate improved liver

function. Alkaline phosphatase is the marker enzyme for plasma and endoplasmic reticulum [33-34] and its decrease indicates the improved synthetic activity of liver, but no such significant effect was observed in case of ALP. From the bilirubin and serum enzyme observations, it seems that KPR increases the liver function significantly (Table 3).

Table 2: Effect of chronic administration of KRP (100 mg/ kg body weight) on Lipid Profile of rats' plasma

Parameters	Male Rats			Female Rats		
	Control (n= 10)	Test (n= 10)	P values	Control (n= 10)	Test (n= 10)	P values
Triglycerides	96.47 ± 1.111	56.142 ± 2.008	0.001***	97.967 ± 3.595	49.667 ± 1.049	0.001***
Total cholesterol	68.18 ± 1.696	58.484 ± 1.635	0.001***	75.244 ± 1.642	64.447 ± 1.653	0.001***
VLDL	14.75 ± 0.761	14.341 ± 0.616	0.829	17.744 ± 0.439	17.524 ± 0.846	0.911
LDL	19.1 ± 0.773	16.006 ± 0.670	0.012*	19.656 ± 0.698	17.573± 0.621	0.043*
HDL	33.01 ± 0.882	30.363 ± 0.816	0.036*	34.378 ± 1.012	29.187 ± 1.108	0.001***

Table 3: Effect of chronic administration of KRP (100 mg/ kg body weight) on various parameters of Liver Functions of rats' plasma

Parameters	Male Rats			Female Rats		
	Control (n= 10)	Test (n= 10)	P values	Control (n= 10)	Test (n= 10)	P values
Total protein	5629.099 ± 65.891	6019.870 ± 87.896	0.001***	5384.664 ± 160.435	6551.382 ± 110.478	0.001***
Albumin	4517.12 ± 117.607	5219.766 ±103.446	0.001***	4221.304 ± 75.562	4960.349 ± 89.305	0.001***
Bilirubin	0.1237 ± 0.00246	0.106 ± 0.006	0.003**	0.0722 ± 0.004	0.0562 ± 0.005	0.001***
sGPT	60.27 ± 0.126	53.092 ± 0.086	0.001***	50.167 ± 0.143	36.620 ± 0.097	0.001***
sGOT	101.73 ± 0.302	68.328 ± 0.216	0.001***	82.500 ± 0.204	56.452 ± 0.264	0.001***
ALP	43.56 ± 0.109	44.184 ± 0.161	0.561	35.456 ± 0.104	34.200 ± 0.171	0.327

Kidney Function

Creatinine content, major kidney function parameter, in the male and female plasma was decreased significantly (male. p = 0.013, female. p = 0.001) but the content of uric acid was not changed in significant manner. This reduced creatinine level might have results from the decreased synthesis or increased functional capacity of tubular excretion [35-36] (Table. 4). Urea content, another kidney function parameter was increased significantly in both of the sexes (male. p = 0.038, female. p = 0.001).

Table 4: Effect of chronic administration of KRP (100 mg/ kg body weight) on various parameters of Kidney Functions of rats' plasma

Parameters	Male Rats			Female Rats		
	Control (n= 10)	Test (n= 10)	P values	Control (n= 10)	Test (n= 10)	P values
Creatinine	0.949 ± 0.012	0.765 ± 0.0325	0.013*	0.978 ± 0.041	0.794 ± 0.023	0.001***
Urea	65.862 ± 1.045	60.751 ± 1.301	0.038*	57.533 ± 1.242	50.093 ± 0.865	0.001***
Uric acid	2.578 ± 0.055	2.611 ± 0.038	0.098	2.797 ± 0.094	2.908 ± 0.0538	0.088

CONCLUSION

The present investigation has shown that KPR improved liver synthetic activity, reduced lipids level and increased kidney function parameters. Diabetic condition alters these parameters specially lipid profile. Abnormalities in serum lipids are associated with diabetes [37-38]. Therefore, KPR will be especially very useful in chronic diarrhea dysentery and fever for those patients who have diabetes also. If further investigations show hypoglycemic activity then KPR could be a safe Complimentary and Alternative Medicine (CAM) for not only chronic diarrhea dysentery and fever but also for diabetes.

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