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Studies of Various Biochemical Parameters of Rat Plasma Following Chronic Administration of "Rohitakarista"-An Ayurvedic Formulation

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Abstract: The study was carried out to investigate the safety profile as well as the effect of "Rohitakarista" (RHT) on various biochemical parameters of rats' plasma after chronic administration. RHT, a classical Ayurvedic preparation used in hepatosplenic disorders, was administered per oral route at a dose of 100 mg kg^{-1} body weight, once daily, up to 46 days for all the experiments. Forty albino rats (*Rattus novergicus*: Sprague-Dawley strains), equally of both sexes, were randomly grouped into four where each group had ten animal/sex. One male and one female group were used as control and other groups were used as test. In the male, rats there was a statistically insignificant increase ($p = 0.763$) in the total protein but there was a statistically significant increase ($p = 0.022$) in the total protein content of the plasma of female rats. Statistically very high significant increase (male: $p = 0.001$ and female: $p = 0.001$) in the albumin content of the plasma was noted in both sexes. In case of bilirubin, interestingly it was decreased very high significantly ($p = 0.001$) in plasma of male rats but increased very high significantly ($p = 0.001$) in the plasma of female rats. In the male rats, statistically there was a very high significant decrease (sGPT: $p = 0.001$, sGOT: $p = 0.001$ and ALP: $p = 0.001$) in the sGPT, sGOT and ALP activities in the plasma. On the other hand, statistically there was a very highly significant increase (sGPT: $p = 0.001$, sGOT: $p = 0.001$ and ALP: $p = 0.001$) in the sGPT, sGOT and ALP activities in the plasma of female rats. Very high significant decrease (male: $p = 0.001$ and female: $p = 0.001$) in creatinine in plasma of both sexes were observed after chronic administration of RHT. Urea in the plasma was decreased very high significantly ($p = 0.001$) in plasma of male rats but increased very high significantly ($p = 0.001$) in the plasma of female rats. There was high significant increase ($p = 0.002$) in uric acid in male rats. On the contrary, no significant increase ($p = 0.324$) of uric acid was observed in female rats.

Key words: Rohitakarista, RHT, kidney function, liver function, ayurvedic, biochemical parameters

INTRODUCTION

"Rohitakarista" is an Ayurvedic OTC drug and traditionally used in pliha (diseases of spleen); udara (diseases of the abdomen); gulma (localised abdominal swelling or tumour); grahani (malabsorption syndrome or sprue) and asthila (enlarged prostate). Basically it is the preparation of *Tecomella undulate* (Family: Bignoniaceae) stem and bark but some other medicinal plants are also used in small proportions (Table 1). *T. undulata*, commonly known as Rohitaka or Rohida, is an important deciduous, ornamental tree found in the Thar Desert. It is also indigenous to western part of India and southeastern parts of Pakistan (Bhau *et al*., 2007). Plant parts are effective against syphilis and eczema and the bark have mild relaxant, cardio tonic and chloretic activities (Bhau *et al*., 2007). Azam (1999) reported that *T. undulata* leaves contain oleanolic acid, ursolic acid and betulinic acid which are strong HIV prohibitors. B sitosterol, triacontanol, cirsimaritin, cirilineol, pentatriacontanol and 4,5-dihydroxy-3,6,8-trimethoxy flavone are other compounds isolated form the leaves of *T. undulata*. It's antibacterial activities (Parckh and Chanda, 2007) and analgesic properties (Ahmad *et al*., 1994) are also reported. Among other plants used in this formulation, *Embelica officinalis* (Euphorbiaceae), *Terminalia chebula* (Combretaceae) and *Terminalia beleracia* (Combretaceae) are medicinally important. Their equi-proportional mixture, known as Triphala in Ayurvedic system, is reported by Jagetia *et al*. (2004) for free radical scavenging and radio-protective activity. But no report on the biochemical study of this formulation is available. Considering the widespread use of Ayurveda as the popular form of TM in Bangladesh, one cannot emphasize enough the need for establishing the safety profiles of Ayurvedic drugs. Keeping in mind the present scenario,

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Table 1: The plants and ingredients used in the formulation of Rohitakarista (RHT) and their therapeutic uses

Name of plants/ Ingredients	Used parts	Botanical name	Family	Amount used	Therapeutic uses
Rohitaka	Stem and Bark	*Tecomella undulata*	Bignoniacea	4.800 kg	Used in Syphilis and Spleen diseases (Parekh, 2007).
Water for decoction				49.152 L	
Reduced to				12.288 L	
Guda (Molasses or Brawn sugar)				9.600 kg	
Amalaki	Fruit powder	*Embelica officinalis*	Euphorbiaceae	48 g	Diuretic, Carminative, Stomachic, Laxative (Ghani, 2003)
Bibhitaka	Fruit powder	*Terminalia beleracia*	Combretaceae	48 g	Laxative, Antipyretic, used in Leprosy, Biliousness, Dyspepsia (Ali, 1998)
Cavya	Stem	*Pipper retrofractum*	Piperaceae	48 g	Anthelminthic, Carminative, Stimulant (Evans, 2002)
Citraka	Root	*Plumbago zeylanica*	Plumbaginaceae	48 g	Sudorific, Antipyretic, used in Dyspepsia, Diarrhoea, Rheumatism, Parasitic skin diseases (Ghani, 2003)
Dhataki	Flower	*Woodfordia fruticosa*	Lythraceae	768 g	Astringent (Evans, 2002)
Ela	Sod	*Elettaria cardamomum*	Scitaminaceae	48 g	Stimulant, Stomachic, Carminative, Diuretic (Ali, 1998)
Haritaki	Fruit powder	*Terminalia chebula*	Combretaceae	48 g	Anti ulcerant, used as Dentifrices (Ali, 1998)
Pippali	Fruit	*Pipper longum*	Piperaceae	48 g	Analgesic, Carminative, Sedative, used in Cough, Bronchitis, Asthma (Ali, 1998)
Pippali mula	Root	*Pipper longum*	Piperaceae	48 g	Analgesic, Carminative, Sedative, used in Cough, Bronchitis, Asthma (Ali, 1998)
Rohitaka patra	Leaf	*Tecomella undulata*	Bignoriacea	48 g	Used in Syphilis and Spleen diseases (Parekh, 2007).
Sunthi	Rhizome	*Zingiber officinale*	Zingiberaceae	48 g	Aromatic stimulant, Carminative, used in Dyspepsia, Flatulent colic, Vomiting spasm, Cold, Cough, Asthma (Ali, 1998)
Tvak	Stem and Bark	*Cinnamomum zeylanicum*	Lauraceae	48 g	Pungent, Aromatic, Astringent, Stimulant, Carminative (Ali, 1998)

this research work on Ayurvedic formulation, Rohitakarista (RHT) 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.

MATERIALS AND METHODS

Dose and route of administration: For the biochemical study, Rohitakarista (RHT) was collected from Sri Kundeswari Aushadhalaya Ltd., Chittagong in November, 2006. The liquid were administered at a volume such that it would permit optimal dosage accuracy without contributing much to the total increase in the body fluid. The drugs were administered per oral route at a dose of 100 mg kg^{-1} body weight for all the experiments. Ketamine were administered intra-peritoneally (500 mg kg^{-1} i.p.).

Experimental animal: Forty eight-week old albino rats (Rattus novergicus: Sprague-Dawley strain) of both sexes, 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 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 30×20×13 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.

Controls: 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 4 groups of 10 animals/sex. Thus 10 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. Administration of the extract was between the hours of 10 am and noon.

Blood samples preparation: At the due of the 46 day treatment period, the animals were fasted for 18 h and also 24 h after the last administration, the animals were anaesthetized using i.p. Ketamine (500 mg kg^{-1} i.p.). 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 h 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), alamine aminotransferase (ALT) and alkaline phosphatase (ALP).

Total protein content of the samples was assayed by the Biuret method (Plummer, 1971). Serum albumin concentration was determined using the method of Doumas *et al.* (1971). 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). Alkaline phosphatase activities were determined using the method as described by King and King (1954).

The absorbance of all the tests was determined using spectrophotometer (UV-Visible Spectrophotometer Model No. UV-1601 PC.). The obtained data were analysed using unpaired t-test according to Glasnapp and Poggio (1985) and presented as Mean±SEM (Standard Error of the Mean). SPSS (Statistical Package for Social Science) for windows was applied for the analysis of data. $p = 0.05$ was taken to be level of significance.

RESULTS AND DISCUSSION

In the male rats there was a statistically insignificant increase ($p = 0.763$) in the total protein but there was a statistically significant increase ($p = 0.022$*) in the total protein content of the plasma of female rats (Table 2). Statistically a very high significant increase (male; $p = 0.001$ and female; $p = 0.001$) in the albumin content of the plasma was noted in both sexes which indicating abnormal liver function (Naganna, 1989). This may be a result of increase synthetic function of liver (Naganna, 1989). In case of bilirubin, useful in differential diagnosis of jaundice from bilirubin overproduction, interestingly it was very high significantly decreased ($p = 0.001$) in plasma of male rats but very high significantly increased ($p = 0.001$) in the plasma of female rats. This result may be sex dependent. The observed elevation in bilirubin in plasma of female rates indicates either abnormal liver function due to jaundice or decrease excretion to bile. In the male rats, there was a statistically very high significant decrease (sGPT: $p = 0.001$, sGOT: $p = 0.001$ and ALP: $p = 0.001$) in the sGPT, sGOT and ALP activities in the plasma. On the other hand, there was a statistically very highly significant increase (sGPT: $p = 0.001$, sGOT: $p = 0.001$ and ALP: $p = 0.001$) in the sGPT, sGOT and ALP activities in the plasma of female rats. Alkaline phosphatase is the marker enzyme for plasma and endoplasmic reticulum (Wright and Plummer, 1974; Shahjahan *et al.*, 2004). The increased alkaline phosphatase activity may be resulted from either de novo synthesis of enzymes molecules or loss of other protein from tissue (Wright and Plummer, 1974; Umezawa and Hooper, 1982). Such increase in alkaline phosphatase activities may results abnormal cellular damage. However, decrease of alkaline phosphatase indicating increased synthetic activity of liver.

There was very high significant decrease (male; $p = 0.001$ and female; $p = 0.001$) in creatinine in plasma of both sexes after chronic administration of RHT. Creatinine is the kidney function parameter which providing a rough approximation of glomerular filtration (Table 3). This very high significant decrease might have results from the decrease synthesis or increased functional capacity of

Table 2: Effect of chronic administration of RHT (100 mg kg^{-1} body weight) on various parameters of liver functions of rats' plasma

	Male rats			Female rats		
Parameters	Control (n = 10)	Test (n = 10)	p-values	Control (n = 10)	Test (n = 10)	p-values
Total protein	5629.0990±65.8914	5655.1290±53.5245	0.763	5384.6644±160.4354	5840.9389±35.4825	0.022*
Albumin	4517.1200±117.6067	5242.3930±15.0939	0.001***	4221.3044±75.5618	5566.1956±27.2487	0.001***
Bilirubin	0.1237±0.002463	0.0132±0.0007377	0.001***	0.0722±0.004006	0.09556±0.002422	0.001***
sGPT	60.2700±0.1257	56.9700±0.03000	0.001***	50.1667±0.1434	57.4333±0.1000	0.001***
sGOT	101.7300±0.3015	95.5100±0.1337	0.001***	82.5000±0.2041	97.4222±0.1372	0.001***
ALP	43.5600±0.1087	42.5600±0.1056	0.001***	35.4556±0.1042	42.1222±0.1869	0.001***

*Significant, ***Very high significant

Table 3: Effect of chronic administration of RHT (100 mg kg^{-1} body weight) on various parameters of kidney functions of rats' plasma

	Male rats			Female rats		
Parameters	Control (n = 10)	Test (n = 10)	p-values	Control (n = 10)	Test (n = 10)	p-values
Creatinine	0.9487±0.01214	0.7925±0.01121	0.001***	0.9778±0.04134	0.7694±0.02074	0.001***
Urea	65.8620±1.0452	47.3650±0.2046	0.001***	57.5333±1.2423	67.7022±0.2755	0.001***
Uric acid	2.5780±0.05481	2.9560±0.08716	0.002**	2.7967±0.0944	2.9033±0.04570	0.324

**High significant, ***Very high significant

tabular excretion (Mitchell *et al*., 1972; Zilva *et al*., 1991). Urea in the plasma was very high significantly decreased ($p = 0.001$) in male rats but was very high significant increased ($p = 0.001$) in of female rats. There was high significant increase ($p = 0.002$) in uric acid in male rats. On the contrary, no significant increase ($p = 0.324$) of uric acid was observed in female rats.

The result of the present investigation has shown that RHT has altered the biochemical parameters investigated. It has found that albumin was increased very high significantly and creatinine was decreased very high significantly in both of the sexes. But interesting sex dependency has found in case of bilirubin, sGPT, sGOT, ALP and urea. All these parameters were very high significantly decreased in male rats but very high significantly increased in female rats. Further study may be carried out to identify the insight of this sex dependency.

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