TOXICOLOGICAL STUDIES OF "CHONDROKOLA ROSH", AN AYURVEDIC PREPARATION ON LIVER FUNCTION TESTS OF RATS.

S. Nasrin[*1], S. C. Bachar[2] and M. S. K. Choudhuri[1]

[1]Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh
[2]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.
***Email:** sweety.rasel@gmail.com

Abstract

Chondrokola Rosh (CKR) is a traditional metallic Ayurvedic preparation widely used by the rural and ethnic people of Bangladesh in dysuria. It is a preparation of various roasted metals (Hg and Cu), non-metal (sulphur and Mica) and medicinal herbs. Considering the controversy over the risk of toxic heavy metals in Ayurvedic herbo-mineral preparations, toxicological parameters on liver functions were investigated. A single dose of 100mg/kg body weight of the preparation was administered orally to the rats of both sexes for ninety days. In this evaluation a statistically significant ($p<0.001$) increase of serum albumin levels in male (17%) and female (15%) rat groups were observed. On the other hand, the plasma bilirubin level was decreased 50% and 28% respectively in both rats groups. But no remarkable differences were observed in plasma protein, sGPT, sGOT and ALP activities from their corresponding control values. This study showed that CKR had no remarkable toxic effect on liver of the animals despite the presence of traces of transformed heavy metals.

Keywords: Chondrokola Rosh, Ayurvedic medicines, Liver function, Toxicological characteristic, Enzymatic activity, Ethnic people.

Introduction

Rasa shastra (Vedic chemistry), branch of Ayurveda, describes the use of metals, gems, minerals and even poisons for manufacturing special formulations to combat chronic and difficult diseases. Several metallic preparations with organic macromolecules termed "bhasmas", in Ayurvedic literature, are employed in the treatment of a variety of disorders (Patel, 1986). Bhasma preparations involve the conversion of the metal into its mixed oxides, during which, the zero valent metal state is converted to a higher oxidation state. The significance of this "Bhasmikarana" is that the toxic nature of the resulting metal oxide is completely destroyed while introducing the medicinal properties into it (Wadekar et al., 2005). Chondrokola Rosh (CKR) is one of unique metallic-herbal Ayurvedic preparations used as alternative medicines among Bangladeshi peoples. In this preparation various roasted metals (bhasma) are used with other medicinal plants. These roasted metals are found to be chelated with organic ligands derived from these plants liquids. These bhasmas are biologically produced nanoparticles and are taken along with herbal liquids. Thus this makes these elements easily assimilable, eliminating their harmful effects and enhancing their biocompatibility (Kumar et al., 2006). As a traditional alternative medicine, CKR is used in dysuria or 'mutrakrcchra' in Sanskrit (BNF of Ayurvedic medicine, 1992). In medicine, specifically urology, dysuria refers to any difficulty in urination. It is sometimes accompanied by pain. It is most often a result of an infection of the urinary tract. In men, the urinary system overlaps with the reproductive system, so dysuria can also be a result of genital infection (Barwitz, 2003). The metals used in this preparation are mercury and copper but non metal includes mica and sulphur. All these were used in roasted form which is produced by a special type of Ayurvedic technique. It is believed that heavy toxic metals such as mercury used in traditional medicine act as a catalyst, which stimulate activity by their presence in the intestines without ever reaching the blood stream (Kumar et al., 2006). Among these, copper is found to have some pharmacological activities. Tamra vshma (roasted copper) has hepatoprotective activity (Tripathi and Singh, 1996). Pharmacological investigations have reported the use of tamra bhasma for treating gastric ulcers and secretion, the management of lipid peroxidation in the liver of albino rats and as an antioxidant (Tripathi and Singh, 1996; Sanyal et al., 1982; Pattanaik et al., 2003). Abhra bhasma (mica/siliceous encrustation) was believed to be a rejuvenator and increase vitality; where gandhaka (sulphur) has some anti-inflammatory and immuno-stimulant activities (Dayrens et al., 1983). In case of suta parada (mercury) it used in several Ayurvedic formulations along with medicinal herb(s), which detoxify the toxicity of mercury.

Mercury is an environmental and industrial contaminant known for its toxicity causing minimata disease (Odell and Sunde, 1997). Its toxic effects were said to be neutralized in the presence of sulphur ((Kumar et al., 2006). It is believed that heavy toxic metals such as mercury used in traditional medicine system act as a catalyzer, which stimulate activity by their presence in the intestines without ever reaching the blood stream ((Kumar et al., 2006). Bhasmas, herbal preparations of

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Ayurvedic origin, contain heavy metals in traces. Very little published information is available on the preclinical toxicity or mutagenicity of these bhasmas (Sathya et al., 2009). Considering the recent controversy over the risk of toxic heavy metals in Ayurvedic herbo-mineral preparations, the present study deals with the toxicological evaluation of Chondrokola Rosh (CKR), a traditional Ayurvedic preparation, on liver functions tests using male and female rats after chronic administration.

Materials and Methods

Drugs, Chemicals and Reagents

For the toxicological study, Chondrokola Rosh (CKR) was collected from Sri Kundeswari Aushadhalaya Ltd, Chittagong. All other reagents, assay kits and chemicals used in this work were purchased from Sigma Chemical Co. St Louis, MO, USA.

Preparation of Sample

Chondrokola Rosh is included in the Bangladesh National Formulary of Ayurvedic Medicine (BNFAM) 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). It is prepared following the method mentioned in the BNFAM 1992 and list of ingredients used in the preparation is mentioned in Table 1.

Experimental Animals

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. 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, Jahangirnagar University.

Experimental Design

In all the experiment a total of forty rats of both sexes were used. The rats were divided into four groups of ten animals where two were male groups and other two were female groups. For both of the sexes, one group was treated with CKR 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 ninety days. For all the pharmacological studies the drugs were administered per oral route at a dose of 100mg/kg body weight. After acclimatization, CKR preparation was administered to the rats by intra-gastric syringe between the 10 am and 12 noon daily throughout the study period.

Blood Samples Collection and Preparation of Plasma

At the end of ninety days treatment, after 24 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 heparinised tubes immediately. Blood 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 24 h of sample collection.

Determination of Biochemical Parameters

Biochemical analysis was carried out on serum, to assess the state of the liver. Biochemical studies involved analysis of parameters such as total protein, serum albumin, bilirubin (total and direct), serum glutamic pyruvic transaminase (sGPT), serum glutamic oxaloacetic transaminase (sGOT) 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). Serum bilirubin is determined according to the method of (Evelyn and Malloy, 1938). Serum glutamic pyruvic transaminase (sGPT), serum glutamic oxaloacetic transaminase (sGOT), and alkaline phosphatase (ALP) were determined by following the method of (King and King, 1954). The absorbances of all the tests were determined using spectrophotometer (UV-Visible Spectrophotometer Model No. UV-1601 PC.).

Statistical Analysis

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The data were analyzed using unpaired t-test as described by (Glasnapp and Poggio, 1985) and expressed as mean ± SEM (Standard Error of the Mean). SPSS (statistical package for social science) for windows (ver-11) was applied for the analysis of data and $p<0.05$, $p<0.01$, $p<0.001$ was taken as the level of significance.

Result and Discussion

Chondrokola Rosh (CKR), a traditional Ayurvedic herbo-mineral preparation widely used by the rural people of Bangladesh in dysuria. It is a preparation of various roasted metals (Hg and Cu), non-metal (Sulphur and Mica) and medicinal plants. The toxicological parameters of this Ayurvedic herbo-mineral preparation were investigated on liver functions tests. A single dose of 100mg/kg body weight of the CKR preparation was administered orally to the male and female rats in separate groups for ninety days. At the end of this chronic study, the serum samples were collected and the toxicological parameters were determined.

Table-1: Name of the ingredients/herbs used in the preparation of "Chondrokola rosh"(CKR).

Name of plants/ ingredients	Used parts	Botanical/scientific name	Family	Amount used
Suta parada	---------	Mercury	--------	12 gm
Tamra bhasma	---------	Roasted copper	--------	12 gm
Abhraka bhasma	---------	Mica or siliceous encrustation	--------	12 gm
Gandhaka	---------	Sulphur	--------	24 gm
Musta (kvatha)	Rhizome	*Cyperes rotandus*	Cyperaceae	Q.S (for bhavna seven times)
Dadima (svarsasa)	Leaf	*Punica granatum*	Puniacaceae	Q.S (for bhavna seven times)
Durva	Root	*Cynodon dactyton*	Poaceae	Q.S (for bhavna seven times)
Ketaki stanaja drava	Flower	*Pandanus odorifer*	Pandanaceae	Q.S (for bhavna seven times)
Sahadevi vari	Whole plant	*Vernonia cineria* (Less)	Asteraceae	Q.S (for bhavna seven times)
Kumari vari	Leaves	*Aloe barbadensis*	Liliaceae	Q.S (for bhavna seven times)
Parpata vari	Whole plant	*Hedyotis corymbosa* L.	Rubiaceae	Q.S (for bhavna seven times)
Ramasitalika toya (rasa)	Whole plant	*Amaranthus tricolor* L	Amaranthaceae	Q.S (for bhavna seven times)
Satavari rasa	Root	*Asparagus racemosus*	Liliaceae	Q.S (for bhavna seven times)
Tikta (katuka)	Rhizome	*Picrorhiza kurroa*	Scrophulariaceae	12 gm
Guduci satva	Stem	*Tinospora cordifolia*	Menispermaceae	12 gm
Usira	Root	*Vetiveria zizanioides*	Graminae	12 gm
Madhavi	Flower	*Hiptage benghalensis*	Malpighiaceae	12 gm
Srigandha	wood	*Santalum album*	Santalaceae	12 gm
Sariva	Root	*Hemidesmus indicus*	Asclepiadaceae	12 gm
Draksa phala	Dry fruit	*Vitis vinifera* L	Vitiaceae	Q.S (for bhavna seven times)

In the study, the total protein content in the plasma was increased in both the control and CKR treated male rats. The result showed no significant difference between the control and the CKR treated groups. But the albumin content was significantly ($p<0.001$) increased (17%) in CKR treated male rats. In the female rats group the total protein and the albumin content in the plasma were also increased in comparison to their control groups. A statistically significant ($p<0.001$) increase was noted (15%) only in the case of albumin for female rats (Table 2). These proteins are important liver function markers. Plasma albumin is well known to decrease in response to inflammation (Benoit et al, 2000). So CKR has no toxic effect on liver. Also the liver function test was performed to asses the state of the liver by the determination of plasma bilirubin level in the rats. After chronic administration of CKR to the male rats, a statistically significant ($p<0.001$) decrease of bilirubin level 50.07% in the plasma was recorded in comparison to their control group. Here, the test medicine also decreased the bilirubin level by 28.12 % in female rats group. The decreased level of bilirubin in plasma indicated lack of toxic effect on liver even after chronic administration of Chondrokola Rosh (CKR). According to (Naganna, 1989), increase in bilirubin indicates the abnormal liver

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function which may be the results of higher synthetic function of the liver. So decrease level of bilirubin in CKR treated rat is indicating the normal liver function. Also test was done to find out the effect of Chondrokola rosh on liver enzymes like- serum glutamic pyruvic transaminase (sGPT), serum glutamic oxaloacetic transaminase (sGOT), and on alkaline phosphatase. In the male rats no significant increase of sGPT activities (1%) in the plasma was observed in comparison to control rat group. Similarly no other changes in case of the sGOT and ALP levels in the plasma were exhibited from their corresponding control values. Alkaline phosphatase is the marker enzyme for plasma and endoplasmic reticulum (Wright and Plummer, 1974; Shahjahan et al., 2004) and its decrease indicates the improved synthetic activity of liver (Table-2). Metallic herbal preparations offer advantages over plant drugs by virtue of their stability over a period, lower dosage, easy storability and sustained availability and contain minerals and metals as integral part of the formulations (Kumar et al., 2006)). They are used with the intention to give therapeutic efficacy to the product of the designated illness.

Table- 2: Effect of chronic administration of CKR (100 mg/kg body weight) on various parameters of Liver function of rat's plasma.

Parameter	Male Rats			Female Rats		
	Mean ± SEM		% Changes	Mean ± SEM		% Changes
	Control (n=10)	Test (n=10)		Control (n=10)	Test (n=10)	
Total protein	5668.09 ±113.64	5826.66 ± 96.87	(2.79%) ^{NS}	5587.54± 109.13	5967.47± 127.39	(6.79%) ^{NS}
Albumin	4566.42 ±106.89	5347.54 ± 87.38	(17%)***	4467.81 ± 82.92	5142.14 ± 79.37	(15%)***
Bilirubin	0.13 ± 0.0039	0.06 ± 0.0033	(50.07%)***	0.11 ± 0.004	0.08±0.004	(28.12%)***
sGPT	61.95 ± 0.13	62.62 ± 0.12	(1%)*	58.58 ± 0.14	59.78 ±0.048	(2%)**
sGOT	111.34 ± 0.28	112.98 ± 0.39	(1.47%) ^{NS}	104.93 ± 0.22	106.39 ± 0.36	(1.38%)*
ALP	44.91 ±0.10	44.74 ± 0.11	(0.37%) ^{NS}	42.20 ± 0.11	42.06 ± 0.12	(0.33%) ^{NS}

In each group 10 male and 10 female rats were taken, P values were calculated using unpaired t-test in comparison to control. *P<0.05, **p<0.01,***p<0.001, NS=Not Significant.

In conclusion the present investigation has shown that CKR reduced bilirubin level and increased albumin levels in both rat groups. At the same time no remarkable changes in the enzymes such as sGOT, sGPT and ALP were observed. It also emphasizes that the mere presence of a chemical compound of metallic origin does not contribute to the toxicity of the finished product as the standard manufacturing process inflicts intense changes and components of herbal origin after sequential reactions with diverse components of processing is responsible for the therapeutic action. It also shows that Chondrokola Rosh (CKR) has no remarkable toxic effect on the liver function of the animals despite the presence of traces of transformed toxic heavy metals.

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