

## Toxicological studies of Rasnairandadi Kvatha Curna

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### Abstract

The present study was undertaken to investigate the toxicological effects of Rasnairandadi Kvatha Curna (RAS), a classical ayurvedic preparations used as an analgesic drug. Various biochemical parameters such as total protein, serum albumin, bilirubin, creatinine, and liver enzymes i.e. serum glutamic pyruvic transaminase (sGPT), serum glutamic oxaloacetic transaminase (sGOT) and alkaline phosphatase (ALP) of experimental rats were analysed after chronic administration of RAS. The total protein content showed no significant difference between the control and the RAS treated both male and female rats. Albumin content significantly increased ( $p < 0.001$ ) in both RAS treated male and female compared to control. There was a significant increase of sGPT ( $p < 0.001$ ) and sGOT ( $p < 0.05$ ) activities found in the plasma of RAS treated male rats compared to control. Also, ALP levels significantly ( $p < 0.001$ ) increased in the plasma of RAS treated male rats. On the contrary, RAS treated female rats revealed a significant decrease in the sGPT and sGOT levels in the plasma. The ALP levels in the plasma of treated female rats were also decreased but was statistically insignificant ( $p = 0.438$ NS) in comparison to their corresponding control values. The level of bilirubin was decreased in male and increased in female rats and it was statistically significant ( $p < 0.001$ ). Overall, the results show some alterations on liver and kidney parameters of experimental rats which indicate that RAS may produce toxic effects.

**Keywords:** Rasnairandadi Kvatha Curna, albumin, bilirubin, creatinine, and glutamic pyruvic transaminase, glutamic oxaloacetic transaminase.

### 1. Introduction

Rasnairandadi Kvatha Curna (RAS) is one of the unique herbal ayurvedic preparation used as alternative medicine in Bangladesh. As an analgesic traditional medicine, it is included 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).

The usual human dose of RAS is 10-15 ml twice a day (Nishteswar *et al.* 2006). Ingredients of RAS preparation has been documented in **Table 1**. Among the ingredients, *Pluchea lanceolata* (Rasna) has anti-inflammatory and analgesic activities and the plant contains high amounts of secondary metabolites quercetin,  $\beta$ -sitosterol, triterpenol which gives it anti-inflammatory and analgesic properties (Ali *et al.* 2001). *Ricinus communis* (Mula) possesses central analgesic activity (Almeida *et al.* 2001). In the Indian system of

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medicine, the leaf, root and seed oil of this plant have been used for the treatment of inflammation and liver disorders (Ivan *et al.* 1998).

**Table 1: Formulary of RAS.**

Ayurvedic Name	Parts Used	Botanical Name
Rasna	Leaf/ Root	<i>Pluchea lanceolata</i>
Eranda (mula)	Root	<i>Ricinus communis</i>
Bala	Root	<i>Sida cordifolia</i> Linn.
Sahacara	Pulp	<i>Baeleria prionitis</i> Linn.
Vari (satavari)	Root	<i>Asparagus racemosus</i>
Dusparsa.(yavasaka)	Pulp	<i>Alhagi pseudalhagi</i>
Vasa (mula)	Root	<i>Adhatoda beddomei</i>
Amrta (guduci)	Stem	<i>Tinospora cordifolia</i>
Devahva (devadaru)	Heart wood	<i>Cedrus deodara</i>
Ativisa	Root	<i>Aconitum heterophyllum</i>
Ghana (musta)	Rhizome	<i>Lithacodia musta</i>
Iksura	Root	kokilaksaka mula
Sathi	Rhizome	<i>Hedychium spicatum</i> Ham.
Visva (sunthi)	Rhizome	<i>Zinziber officinalis</i>

The water extract of the leaves of *Sida cordifolia* (Bala) was reported to possess analgesic and anti-inflammatory activities in mice models (Franzotti *et al.* 2000). *Barleria prionitis* has numerous medicinal properties including stomach disorders, ulcer, fever, migraine, arthritis and gout, internal abscesses, oedema, haemoptysis, urinary infection, seminal disorders and obesity (Khare *et al.* 2004, 2007). *Asparagus racemosus* are considered to be effective as antispasmodic, appetizer, stomach tonic, aphrodisiac, galactagogue, astringent, anti-diarhoeal, antidysenteric, laxative, anticancer, anti-inflammatory, blood purifier, antitubercular, antiepileptic and also in night blindness, kidney problems and in throat complaints (Thomson *et al.* 2002). Decoctions of *Alhagi pseudalhagi* have long been used in folk medicine as a cholegic and astringent for cholitis, gastritis, and stomach ulcers; to prevent dehydration (Sinel-nikov *et al.* 1965) and as an antipyretic (Gurbanov *et al.* 1976). The root, leaves and flowers of *Adhatoda vasica* is used in the form of juice and decoction to treat fever, intrinsic haemorrhage, cough, asthma, consumption, skin diseases, obesity, oedema, skin diseases, leucorrhoea, difficult labour, vomiting, piles, pox, retention of urine, diseases of mouth and as rejuvenative (Roshan *et al.* 2011). *Tinospora cordifolia* (Guduchi) is widely used in veterinary folk medicine/ayurvedic system of medicine for its general tonic, antiperiodic, anti-spasmodic, anti-inflammatory, antiarthritic, anti-allergic and anti-diabetic properties (Nadkarni *et al.* 1976, Kirtikar *et al.* 1975, Chopra *et al.* 1956, 1982; Zhao *et al.* 1991). Recent *in vivo* and *in vitro* studies of *Cedrus deodara* (Roxb.) have indicated its anti-inflammatory, analgesic, anti-hyperglycemia, antispasmodic, insecticidal, anti-apoptotic, anti-cancer, immunomodulatory,

molluscidal, anxiolytic and anticonvulsant properties (Amrendra *et al.* 2011). The rhizome extract of *Hedychium spicatum* are administered for blood purification, bronchitis, indigestion, treatment of eye disease and inflammations (Prakash *et al.* 2001).

The World Health Organization (WHO) has officially recognized and recommended large-scale use of herbal (Unani and Ayurvedic) medicines, particularly in the developing countries, as an alternative system of medicine to deliver health care services at the primary health care level (WHO report 2002). According to WHO, an estimated 1.5 billion people of the world are now getting treatment with these medicines (WHO report 1999a, 1999b). Ayurveda, a traditional system of medicine in Indian subcontinent, have major treatment scopes globally (Mukherjee *et al.* 2009; Bele *et al.* 2010).

Although the Ayurvedic medicine has widespread popularity in Bangladesh, very little exploration has been done on their safety and rational usages. In this context, it is essential to establish the safety profiles of Ayurvedic drugs and this research work on Ayurvedic formulation, Rasnairandadi Kvatha Curna (RAS) was accomplished to explore a spectrum of its toxicological aspects utilizing *in vivo* animal models. The objective is to explore the possible toxicological profile of the drug under study and to some degree justify the use of this drug under the stated circumstances.

## 2. Materials and methods

**2.1. Drugs, chemicals and reagents:** Rasnairandadi Kvatha Curna (RAS) was collected from Sri Kundeswari Aushadhalaya Ltd., Chittagong, Bangladesh and all other reagents, assay kits and chemicals used for the toxicological study in this study were purchased from Sigma Chemical Co. St Louis, MO, USA.

**2.2. Preparation of sample:** The kwath was prepared from dried powder according to the procedure mentioned in Bangladesh National Ayurvedic Formulary (BNAF), 1992. The kwath was prepared by adding 160 mL of distilled water with 5g of the powder and was thoroughly mixed to make a uniform suspension. It was then boiled till the volume was reduced to 40 mL and was finally filtered. This filtrate was collected and marked as collection-I. Then, residue was again boiled with 160 mL of water till the volume was reduced to 40 mL and was then filtered. This filtrate was marked as collection-II. Both the fractions (collection-I and-II) were mixed together and reduced to 20 mL by boiling and this mixture was known as kwath and was used for the targeted toxicological studies. For the toxicological experiments, tested drug (Kwath) was administered at a volume ensuring optimal dosage accuracy without contributing much to total increase in

body fluid. So, the kwath was administered per oral route at a dose of 40 mL/kg of the body weight. The anaesthetic drug ketamine was administered intraperitoneally (500 mg/kg body weight) (Ullah *et al.* 2008).

### 2.3. Experimental animals and their management:

Eight-weeks old Albino rats (*Rattus norvegicus*; Sprague-Dawley strain) of both sexes and bred maintained at the animal house of the Department of Pharmacy, Jahangirnagar University, were used in the toxicological experiments. These animals were apparently healthy and weighed between 50 to 70 g (Ullah *et al.* 2008). 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 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. Prior to the experiment, they were randomly divided into 4 groups of 10 animals/ sex. Another group of rats (consisting of same numbers of animal in each test group) was simultaneously employed in the experiment which were treated with distilled water as placebo as per the same volume as the drug treated group for the same number of days and this group served as the control.

**2.4. Experimental Design:** The Sprague-Dawley rats of both sexes were treated with 40 mL/kg per oral doses of the kwath every day for a period of 45 days. Simultaneously, the control group of rats were treated with equivalent amounts of distilled water for the same period of time. These two groups were referred to as RAS and Control groups respectively. After the completion of the 45 days period, rats of both RAS and control groups were anaesthetized using Ketamine (500 mg/kg i.p.) and after sacrificing, the blood samples were collected from post vena cava and transferred into heparinised tubes immediately (Kundu *et al.* 2012). Blood was then centrifuged at 4,000 rpm for 10 mins using bench top centrifuge (MSE Minor, England) to separate plasma from red blood cells. Plasma samples were separated and were collected using dry Pasteur pipette and stored in the refrigerator for analysis. All

analyses were completed within 24 hrs of sample collection.

### 2.5. Determination of Biochemical Parameters:

Biochemical analysis was carried out on blood serum to assess the state of the liver and kidney. Biochemical studies involved analysis of parameters such as total protein, serum albumin, bilirubin (total and direct), creatinine, and liver enzymes such as 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 1978). Serum albumin concentration was determined using the method developed by Doumas *et al.* (1971). 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 (HDL) cholesterol were determined using Randox Laboratory kit reagents. Serum triacylglycerol level was estimated using Randox Laboratory test kit and Very-low-density lipoprotein 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 developed by Evelyn and Malloy (1938) was employed to determine the serum bilirubin concentration of the samples. The procedure described by Tietz *et al.* (1994) was used to determine serum creatinine concentration. The serum urea concentration was determined by the method developed by Kaplan *et al.* (1965). Alkaline phosphatase activities were determined using the method as described by King *et al.* (1954). The absorbances of all the tests were determined using spectrophotometer (UV-Visible Spectrophotometer, Model No. UV-1601 PC.).

**2.6. Statistical Analysis:** The data were analyzed using unpaired t-test as described by Glasnapp *et al.* (1985) and expressed as mean  $\pm$  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. Differences between groups were considered significant at  $p < 0.05$ , 0.01 and 0.001.

## 3. Results

**3.1. Liver function parameters:** The results of various liver function parameters after chronic administration of RAS are shown in **Table 2**. The results showed that albumin content in the plasma significantly increased ( $p < 0.001$ ) in RAS treated both male and female rats compared to controls. However, the total protein content

**Table 2: Study of the liver function parameters after chronic administration of RAS.**

Parameters	Male rats			Female rats		
	Control(n=10)	Test (n=10)	% change	Control(n=10)	Test(n=10)	% change
<b>Total protein</b>	6045.15±96.02	6219.86±94.67 <sup>NS</sup>	↑ 2.89%	5102.05±125.34	5431.87±139.18 <sup>NS</sup>	↑ 6.46%
<b>Albumin</b>	4814.28±118.94	5642.96±115.41***	↑ 17.21%	3997.00±72.90	4535.52±76.43***	↑ 13.47%
<b>Bilirubin</b>	0.13±0.00	0.08±0.00***	↓ 40.09%	0.09±0.00	0.12±0.00***	↑ 35.01%
<b>sGPT</b>	59.89±0.13	60.9±0.13**	↑ 1.69%	56.01±0.15	54.08±0.08***	↓ 3.43%
<b>sGOT</b>	103.34±0.28	104.65±0.41*	↑ 1.26%	95.59±0.22	92.91±0.21***	↓ 2.80%
<b>ALP</b>	42.47±0.10	44.52±0.09***	↑ 4.83%	39.22±0.11	39.11±0.13 <sup>NS</sup>	↓ 0.27%

Note: NS=Non-significant; ↑= increased; ↓= decreased; \* p<0.05; \*\*p <0.01; \*\*\*p< 0.001.

**Table 3. Effect of RAS on lipid profile after chronic administration of RAS.**

Parameters	Male rats			Female rats		
	Control(n=10)	Test (n=10)	% change	Control(n=10)	Test(n=10)	% change
<b>Triglycerides</b>	93.21±1.61	83.12±1.36***	↓ 10.82%	103.98±3.46	80.12±2.92***	↓ 22.94%
<b>Total cholesterol</b>	67.57±1.56	84.72±0.97***	↑ 25.38%	79.63±1.74	92.85±1.51***	↑ 16.60%
<b>VLDL</b>	15.04±0.63	20.00±0.93***	↑ 32.96%	18.72±0.59	21.52±0.35***	↑ 14.97%
<b>LDL</b>	17.98±0.70	21.55±0.92***	↑ 19.85 %	20.29±0.71	25.68±0.93***	↑ 26.53%
<b>HDL</b>	30.89±0.83	39.54±0.73***	↑ 27.99 %	35.19±1.02	44.38±1.03***	↑ 26.12%

Note: ↑= increased; ↓= decreased; \* p<0.05; \*\*p <0.01; \*\*\*p< 0.001.

**Table 4. Study of kidney function parameters after chronic administration of RAS.**

Parameters	Male rats			Female rats		
	Control(n=10)	Test (n=10)	% change	Control(n=10)	Test(n=10)	% change
<b>Creatinine</b>	0.88±0.02	0.82±0.04 <sup>NS</sup>	↓ 6.96%	1.09±0.04	0.82±0.04***	↓ 25.34%
<b>Urea</b>	59.9±0.97	50.99±1.34***	↓ 14.87%	67.71±1.23	56.64±1.18***	↓ 16.35%
<b>Uric acid</b>	2.95±0.08	2.63±0.06*	↓ 10.93%	2.5±0.08	2.78±0.06***	↑ 11.35%

Note: NS=Non-significant; ↑= increased; ↓= decreased; \* p<0.05; \*\*p <0.01; \*\*\*p< 0.001.

displayed no significant difference between the control and the RAS treated groups in both sex rats. Additionally, bilirubin content in the plasma significantly (p<0.001) decreased (40.09%) in RAS treated male rats compared to their control group. However, in the female rats it was increased (35.01%) significantly (p<0.001) in the plasma (Table 2). Moreover, in the male rats there was a significant (p<0.001) increase of sGPT activities (1.69%), and significant (p<0.05) increase of sGOT activities (1.26%) in the plasma was observed in RAS treated group in comparison to control. Also, ALP levels significantly increased (p<0.001) in the plasma. The RAS treated

female rats revealed highly significant decrease (3.43%) in the sGPT and (2.80%) sGOT levels in the plasma. The ALP level in the plasma for the female rats was also decreased (0.27%) which was statistically insignificant compared to their corresponding control values.

**3.2. Lipid profiles:** The results of various lipid components after chronic administration of the RAS are presented in Table 3. The triglyceride level was decreased significantly (10.82%, p<0.001) in male rats group. Statistically highly significant increase in total cholesterol (25.38%), VLDL (32.96%), LDL (19.85%)

and HDL (27.99%) content of the plasma were observed from their corresponding control values in male rats. In the female experimental rats, triglyceride content in the plasma significantly decreased (22.94%,  $p < 0.001$ ). On the contrary, the total cholesterol, VLDL, LDL and HDL content in the plasma of female rats were increased for 16.60%, 14.97%, 26.53% and 26.12% respectively.

**3.3. Kidney function parameters:** The kidney function analysis includes the study of creatinine, urea and uric acid and the results are presented in **Table 4**. The data showed that in the male rats there was a decrease in both the creatinine and the urea content in the plasma. But statistically very highly significant ( $p < 0.001$ ) decrease (14.87%) was noted only in the case of the urea content in the plasma. In the female rats, there was a statistically significant ( $p < 0.001$ ) decrease in both the creatinine and the urea content in the plasma. About 25.34% and 16.35% decrease ( $p < 0.05$ ) in plasma creatinine and urea respectively were observed for RAS treated female rats in comparison to their control. In contrast, the same group of rats showed significant increase ( $p < 0.001$ ) concentration of uric acid (11.35%) level compared to control.

#### 4. Discussion

The various plasma proteins and enzymes levels are the markers of liver functions. The analysis of experimental data showed no significant difference between the control and the RAS treated groups for their total protein content. But, the albumin contents were significantly ( $p < 0.001$ ) increased in both RAS treated male and female rats. These proteins are important liver function markers (Johnstone *et al.* 1999) and the plasma albumin is well known to decrease in response to inflammation (Benoit *et al.* 2000). Additionally, the plasma bilirubin level was also analyzed to assess the state of the liver. After chronic administration of RAS to the male rats, there was a statistically significant decrease in the bilirubin content in the plasma compared to their control group. The decreased level of bilirubin in plasma indicated lack of toxic effect on liver even after chronic administration of RAS. So, decreased level of bilirubin in RAS treated rat indicates the normal liver function in male rats. However, in the female rats, there was a statistically highly significant increase in the bilirubin content in the plasma. According to Naganna *et al.* 1989, the increase of bilirubin content indicates the abnormal liver function which may be due to over synthetic function of the liver. Hepatic cells participate in a variety of metabolic activities and contain a host of enzymes. In the tissue, AST and ALT are found in higher concentrations in the cytoplasm and ALT in particular also exists in mitochondria (Wells *et al.* 1988).

In the RAS treated male rats, we found significant increase ( $p < 0.001$ ) of sGPT and sGOT ( $p < 0.05$ ) level in the plasma of RAS treated rats compared to control. Also, ALP levels significantly ( $p < 0.001$ ) increased in the plasma. Hepatic cells participate in a variety of metabolic activities and contain a host of enzymes. In tissues, sGOT and sGPT are found in higher concentrations in cytoplasm and sGOT in particular also exists in mitochondria. In liver injury, the transport function of the hepatocytes is disturbed resulting in the leakage of plasma membrane, thereby causing an increased enzyme level in serum, and soluble enzymes like sGOT are also released similarly. The elevated activities of sGOT and sGPT in serum are indicative of cellular leakage and loss of functional integrity of cell membranes in liver (Rajesh *et al.* 2004). Alkaline phosphatase is excreted normally *via* bile by the liver. In liver injury due to hepatotoxin, there is a defective excretion of bile by the liver which is reflected in their increased levels in serum (Singh *et al.* 1998). These results indicated the impaired liver function after the administration of RAS. The RAS treated female rats exhibited a significant decrease in the sGPT and sGOT levels in the plasma. The ALP level in the plasma of RAS treated female rats was also decreased but it was not statistically significant.

As the lipid profile plays a significant role in the treatment of hyperlipidemic patient, the study was designed to assess various parameters associated with that condition. The result showed a significant ( $p < 0.001$ ) decrease of plasma triglyceride level in both RAS treated male and female rats compared to control. Total cholesterol, VLDL, LDL, and HDL content in the plasma were found to be significantly increased in both RAS treated male and female rats which is vulnerable for coronary heart diseases (Mironova *et al.* 2000). VLDL-C is the major transport vehicle for TG from liver to extrahepatic tissues whereas LDL-C is not secreted as such from the liver; rather it seems to be formed from VLDL-C after partial removal of TG by lipoprotein lipase (Mayes *et al.* 1977). Lipoprotein lipase in striated muscle is enhanced in animal on fat rich diet (Kimball *et al.* 1983). Elevated triglyceride is a risk factor for coronary artery disease. In 1959, a case-control study by Albring and Man identified increased fasting triglyceride levels for patients with coronary artery disease compared with control subjects (Kasai *et al.* 2013). The earliest prospective study of triglyceride and ischemic heart disease demonstrated an increased incidence of ischemic heart disease among men with elevated triglyceride levels at baseline compared with men with lower levels (Jorgen *et al.* 1998).

We also found a significant ( $p < 0.001$ ) decrease of urea content in the plasma in RAS treated male rats. In the female rats, there was a significant ( $p = 0.001$ ) decrease of both creatinine and urea content in the

plasma, the major kidney function parameters. The uric acid content significantly decreased ( $p < 0.05$ ) in plasma of RAS treated male rats compared to their control male rats. The female rats showed a significant high concentration of uric acid level ( $p < 0.001$ ). The serum creatinine and urea is elevated whenever there is a significant reduction in the glomerular filtration rate (GFR) or when urine elimination is obstructed. Overproduction of uric acid causes gout and it may also lead to progressive renal insufficiency (Lehninger *et al.* 1993). Hyperuricemia is also associated with Diabetes mellitus (Brenner *et al.* 1987), hypertriglyceridemia and obesity (Safi *et al.* 2004). In renal disease, as the rate of serum urea production exceeds the rate of clearance the serum urea accumulates (resulting in uremia). Other causes of uremia include high protein diet, increased catabolism due to starvation, tissue damage, sepsis and steroid treatment and absorption of amino acids and peptides from digested blood after hemorrhage into the gastrointestinal lumen or soft tissue (Mayne *et al.* 1994).

## 5. Conclusion

From the evaluation of different biochemical parameters in the plasma, it was observed that in most cases RAS showed nontoxicity (total protein, albumin, bilirubin, sGPT, sGOT, ALP, triglycerides, creatinine, urea and uric acid on male rats and total protein, albumin, triglycerides, total cholesterol, HDL, creatinine, urea and uric acid on female rats). At the same time RAS showed some toxicity also (total cholesterol, VLDL and LDL on male rats and bilirubin, sGPT, sGOT, ALP, total cholesterol, VLDL, LDL, uric acid on female rats). This study will provide a better understanding of the toxicological profile of the drug under study and justify the use of this drug in terms of safety under the stated circumstances.

## 6. Ethical approval

The study was approved by the Ethical Review Committee, Faculty of Life Sciences, Jahangirnagar University.

## 7. Conflict of interest statement

We declare that we have no conflict of interest.

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