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A STUDY OF SUBACUTE TOXICITY OF *KAJJALI*, A COMBINATION OF MERCURY AND SULPHUR ON ALBINO RATS

THERASILIN LOUIS[1], P YUVARAJ[2], V MADHAVACHANDRAN[3], NISHANTH GOPINATH[4]

[1] Experimental Pharmacology Unit, Nagarjuna Herbal Concentrates Ltd, Kalayanthani, Thodupuzha, Idukki, Kearla, India

[2] Officer R&D, Experimental Pharmacology Unit, Nagarjuna Herbal Concentrates Ltd Kalayanthani Post, Idukki, Kearla, India

[3] Director NIAR, Nagarjuna Herbal Concentrates Ltd, Kalayanthani Post, Idukki –

[4] Asst. Manager R&D, Nagarjuna Herbal Concentrates Ltd Kalayanthani Post, Idukki, Kearla, India

ABSTRACT

Kajjali is a combination of Mercury and Sulphur, which is used for treatment in Ayurveda for many disorders. This study investigated the sub-acute toxicity of *Kajjali* in rat models. Different doses of *Kajjali* powder suspension were given to the animals for a period of 60 days. The changes in hepatic markers (AST, ALT, ALP, bilurubin, protein and albumin), lipid profile (TC, TG, HDL and LDL), renal markers (urea, uric acid and creatinine) and other behaviors were noted. From the results, it can be concluded that *Kajjali* powder suspension has no acute and sub-acute toxicity.

Key words: *Kajjali*, Toxicity, Mercury, Sulphur

INTRODUCTION

Ayurveda make use of herbal preparations for their curative effects. Use of metallic herbal preparations (*bhasma*), in which a process termed *bhasmikarana* used to prepare the drug, is unique to Ayurveda. It is believed that the *bhasmikarana* process converts metal into its specially desired chemical compound, which eliminates the toxicity of the metal and has the necessary medicinal benefits[1-2].

Kajjali is prepared by purified *Parada* (mercury) and *Gandhaka* (sulphur) ground without adding any liquid till it becomes a homogenous mixture (collyrium, a black soft substance) [3]. *Kajjali* is added with different *Parada yoga* (mercurial formulations) and such formulations are also called as *Kajjali bandha*[4]. *Kajjali* can be prepared by adding *Gandhaka* to *Parada* by half, equal or double in quantities[5]. Internal administration of *Kajjali* is said to cure many disorders, pacifies the *Tridosha* (disorder of the three humours of the body) and acts as *Vrushya* (aphrodisiac). Further, it is also used as *Sahapana* (taking together with the medicine) and *Anupana* (a vehicle taken after the medicine) [6]. In addition, K*ajjali* is one of the prime ingredients in various *Rasayoga* (herbo-mineral formulations) and is also used as a medicine individually. Different proportions of purified *Parada* and *Gandhaka* are mentioned in the preparation of *Kajjali*. As the toxicity of Mercury is known, even the Ayurvedic physicians are

reluctant to use Mercuric compounds as medicine. Therefore, the authors aimed to assess whether any toxic effects caused by *Kajjali*. In this study, the sub-acute toxicity of *Kajjali* on male albino rats was examined.

MATERIAL AND METHODS

2.1. Animals:

Male Wistar rats weighing 220-250 g bred in Nagarjuna Herbal Concentrates Ltd., Thodupuzha, Kerala, India, were used in the present study. The rats were grouped and housed in polyacrylic cages (38 × 23 × 10 cm) with not more than 3 animals per cage and maintained under standard laboratory conditions with natural dark and light cycle. They were allowed free access to standard dry rat/mice diet and water *ad-libitum*. All animal for the experiments were approved by the Institutional Animal Ethics Committee, Nagarjuna Herbal Concentrates Ltd., and were maintained in accordance with the guidelines of the CPCSEA.

2.2. Preparation of *Kajjali*

Equal quantity of Mercury and Sulphur were ground well until it became a very fine black powder, like collyrium and the dazzling particles of Mercury completely disappeared without adding any liquid. The resultant material, which is very soft and fine looking *Kajjala* (a black soft substance which is put in the eyes-collyrium) is called *Kajjali*[7].

2.3. Sub-Acute toxicity:

18 male rats (220±10g) were taken for the study and were classified into group 1, group 2 and group 3, each containing six animals. Group 1 orally received 0.5% of Tween 20 for a period of 60 days, which is served as a normal control. Group 2 orally received 5/mg/kg of *Kajjali* powder suspension for a period of 60 days. Group 3 orally received 10 mg/kg of *Kajjali* powder suspension for a period of 60 days. Behavior, temperature, feces, food intake, body weight and water intake were noted. On 61st day, the animals were sacrificed for biochemical analysis. Then, the internal organs were cut out and the internal organ damages were noted.

2.4. Biochemical analysis:

On 61st day, all the animals in different groups were sacrificed by decapitation (Pentobarbitone sodium anaesthesia, 60 mg/kg body weight), blood was collected and serum was separated by centrifuging and used for various biochemical estimations. The organs were collected and observed for pathological and morphological changes.

2.5. Statistical analysis

The values are expressed as the mean ± SD and the significance between different groups were determined by One-Way-Analysis of Variance (ANOVA) coupled with Duncan's Multiple Range Test (DMRT), taking $p < 0.05$ as significant.

RESULTS AND DISCUSSION

Treatment with *Kajjali* for 60 days at the doses of 5 and 10 mg/kg body weight, p.o., did not cause any obvious toxic symptoms. The general behavior of animals was not changed. Similarly, food and water intake of animals and body weight gain were not significantly altered in the treatment groups, compared to control. State of the fecal droppings was not changed by the 60 days treatment in rats of all groups.

The effect of *Kajjali* on serum biochemical parameters is shown in Table 2.

Serum enzymes ALT, AST and ALP levels, as well as serum total and direct bilirubin, serum protein, albumin, glucose, haemoglobin, urea, uric acid and creatinine levels (Table 1 and 2) were not significantly altered by the *Kajjali* treatment. However, the treatment for 60 days decreased LDL levels whereas HDL levels were not significantly influenced (Table 3). Total cholesterol and triglycerides levels were also decreased at the dose of 5 and 10 mg/kg. In sub-acute toxicity, 10 mg/kg (four times higher than the human dose) was investigated. Even in this dose, the *Kajjali* has shown no toxic effect.

Both adverse drug reactions and poisonings associated with the use of herbal medicines have increasingly been reported[8-10]. Herbal use has been associated with organ toxicities of heart, liver, blood, kidneys, central nervous system, and skin and carcinogenesis[11-17]. In this contest, the present results are interesting. Normal human dose of *Kajjali* powder suspension is 125 mg/50 kg. In sub-acute toxicity, 10 mg/kg (four times higher than the human dose) was investigated and even in this dose, the *Kajjali* has shown no toxic effect.

CONCLUSION

The administration of *Kajjali* suspension did not show any sub-acute toxic signs in the experimental animals in terms of alteration in hepatic markers, renal markers and other behaviors such as food and water intake, body weight, stool, etc. Therefore, this study concluded that the K*ajjali*, at doses investigated, did not provoke toxic effects to the animals' liver, heart and kidney.

Table 1: Effect of *Kajjali* on Haemoglobin, Bilirubin, Protein and Glucose levels in normal rats

Groups	Haemoglobin (g%)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Glucose (mg/dl)	Total protein (g/dl)	Albumin (g/dl)
Normal control	12.0± 0.7 [a]	0.54±0.10 [a]	0.27±0.08 [a]	80.1±7.31 [a]	6.29±0.47 [a]	4.51±0.12 [a]
5 mg/kg	12.3±0.4 [a]	0.51±0.13 [a]	0.20±0.11 [a]	81.6±12.1 [a]	6.05±0.49 [a]	4.64±0.35 [a]
10 mg/kg	**12.7±1.0 [a]**	**0.53±0.22 [a]**	**0.21±0.05 [a]**	**81.7±8.79 [a]**	**6.34±0.39 [a]**	**4.64±0.38 [a]**

Values are given as mean ± S.D.E for six rats in each group. Values not sharing a common superscript letter differ significantly at $p<0.05$

Table 2: Effect of *Kajjali* on ALT, AST, ALP, Urea, Uric acid, and Creatinine in normal rats

Groups	ALT (IU/L)	AST (IU/L)	ALP (KA unit)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
Normal control	44.4±3.7[a]	64.4±8.6 [a]	56.6±3.6 [a]	20.0±2.95 [a]	4.14±1.31 [a]	34.81±0.38 [a]
5 mg/kg	42.4±2.5 [a]	61.3±4.2 [a]	52.4±2.1 [a]	22.5±4.31 [a]	3.35±0.79 [b]	35.97±0.26 [a]

10 mg/kg	42.6±5.0 [a]	62.2±5.1 [a]	59.1±1.5 [a]	19.6±1.31 [a]	3.44±0.73 [b]	33.81±0.22 [a]

Values are given as mean ± S.D.E for six rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05

Table 3: Effect of *Kajjali* on lipid profile in normal rats

Groups	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
Normal control	95.4±4.77 [a]	58.5±9.26 [a]	49.2±5.22 [a]	29.7±1.85 [a]	16.6±9.38 [a]
5 mg/kg	94.0±4.05 [a]	53.7±18.4 [b]	56.1±4.18 [b]	26.1±3.69 [b]	11.7±5.32 [b]
10 mg/kg	96.0±1.73 [a]	58.7±63.7 [a]	57.3±1.43 [b]	27.5±12.7 [ab]	11.4±5.56 [b]

Values are given as mean ± S.D.E for six rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05.at p<0.05.

REFERENCE

1. Kumar A, Nair AGC, Reddy AVR, Garg AN. Availability of essential elements in Bhasma: Analysis of Ayurveda metallic preparations by INAA. J radioanal Nucl Chem, 2006;270:173.
2. Wadeka MP, Rode C V, Bendale YN, Patil KR, Prabhune AA. Preperation and characterization of a copper based Indian traditional drug Tamra bhasma. J Pharm Biomed Anal 2005;39:951.
3. Pandita Kashinath Shastry edited by Rasa Tarangini by Pranacharya Sri Sadananda Sharma published by Motilal anarasidas. New Delhi. Reprint. 2004, 16-17.
4. Ashok D, Satpute Translated Rasaratna Samuccaya Published by Chaukhamba Sanskrit Pratishthan. Delhi. Reprint. 2006; 261.
5. Pandita Kashinath Shastry edited by Rasa Tarangini by Pranacharya Sri Sadananda Sharma published by Motilal anarasidas, New Delhi Reprint. 2004;124-125.
6. Pandita Kashinath Shastry edited by Rasa Tarangini by Pranacharya Sri Sadananda Sharma published by Motilal anarasidas, New Delh Reprint. 2004; 261.
7. Satpute A D, Rasaratna samuchchaya. 2006;181.
8. Deng JF. Clinical and laboratory investigations in herbal poisonings. Toxicol 200;227:571–576.
9. Ernst E, Adverse effects of herbal drugs in dermatology. Br J Dermatol 2000;143:923–929.
10. Klepser TB, Klepser ME, Unsafe and potentially safe herbal therapies. Am J Health Syst Pharm 1999;56: 125–138.
11. Bensoussan A, Myers SP, Drew AK, Whyte I.M, Dawson AH. Development of a Chinese herbal medicine toxicology database. J Toxicol Clin Toxicol 2000;40:159–167.
12. Ernst E. Adverse effects of herbal drugs in dermatology. Br J Dermatol 2000;143:923–929.
13. Kessler DA. Cancer and herbs. N Engl J Med 2000;342:1742–1743.

14. McRae CA, Agarwal K, Mutime D, Bassendine MF. Hepatitis associated with Chinese herbs. Eur J Gastroenterol Hepatol 2002;14:559–562.
15. Stedman C. Herbal hepatotoxicity. Seminars in Liver Disease. 2002;22: 195–206.
16. Villegas JF, Barabe DN, Stein RA, Lazar E. Adverse effects of herbal treatment of cardiovascular disease: what the physician must know. Heart Disease. 2001;3: 169–175.
17. Zaacks SM, Klein L, Tan CD, Rodriguez ER, Leikin J. Hypersensitivity myocarditis associated with ephedra use. J Toxicol Clin Toxicol. 1999;37:485–489.

CORRESPONDING AUTHOR

Dr. Therasilin Louis
M Phil, PhD (Biochemistry)
Officer R&D, Experimental Pharmacology Unit,
Nagarjuna Herbal Concentrates Ltd
Kalayanthani Post, Idukki, Kerala, India
E-mail:dr.therasilin@gmail.com