International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Original Article **CODEN: IJPNL6**

TOXICOLOGICAL STUDIES OF AN AYURVEDIC MEDICINE KANKAYANA GUTIKA

Tanmony Sana[1], Md. Mamun Sikder[1], Paritosh Chakma[1], Marjana Khalil[2], Nayma Abedin[1], Md. Al Foyjul Islam[1], Mohammad Jashim Uddin[3] and M. S. K. Choudhuri[1]*

[1]Department of Pharmacy, Jahangirnagar University, Savar, Dhaka -1342, Bangladesh
[2]Department of Pharmacy, East West University, Dhaka-1212, Bangladesh
[3]Department of Pharmacy, Jessore University of Science and Technology, Jessore-7408, Bangladesh

*Corresponding author e-mail: mskc1954@gmail.com

Received on: 28-02-2017; Revised on: 21-04-2017; Accepted on: 10-06-2017

ABSTRACT

Kankayana Gutika (KKY) have been used for the treatment of gulma (localized abdominal swelling or tumor) in Indian subcontinent. However, safety and efficacy of this treatment have not been evaluated. Therefore, the present study was carried out to evaluate the efficacy and safety of KKY in experimental animals. During this study, various experiments on body growth rate, organ-body weight ratio and tissue hydration indices were performed to evaluate its efficacy and toxicity. To find out the toxicological characteristic of KKY, it was administered chronically to the male Sprague-Dawley rats at a dose of 400 mg/kg for 46 days and the following toxicological changes were noted. All throughout the experimental period the KKY treated animals were always maintaining negligible changes in body weight. There is a statistically very highly significant (p=0.001, 25.4% decrease) decrease in the relative percent weight of the male rat liver. There is also a statistically significant (p=0.026, 35.80% decrease) decrease in the relative percent weight of the male rat thymus.

Keywords: Kankayana Gutika, ayurvedic formulation, percent organ weight, organ water content.

INTRODUCTION

Ayurveda, the ancient Indian traditional system of medicine, aimed with prevention and cure of diseases as well as promotion of quality of life [1, 2]. Thousands of therapeutics have been described in various classical texts of Ayurveda for the prevention and management of different maladies using numerous plants either single or in combinations. The great scholars of Ayurveda always tried to validate Ayurveda according to that era. Therefore they also used to include new treatment principles, formulations and plants from other system of medicine. This was the generosity of the great *Acharyas* of Ayurveda for the service of humanity. Kankayan Gutika or vati is ayurvedic medicine in tablet form which is used in treatment of Gulma roga. In Ayurveda gulma roga is a condition of localised abdominal tumor, lump or swelling. This medicine is made from blend of many natural herbs like *Hedychium spicatum*, *Inula recemosa*, *Baliospermum montamum*, *Plumbago zeylanica*, *Cajanus cajan*, *Zingiber officinale*, *Acorus calamus*, *Ipomea turpethum*, *Ferula asafoetida*, *Potassium carbonate*, *Garcinia pedunculata*, *Trachyspermum ammi*, *Cumminum cyminum*, *Piper nigrum*, *Coriandrum sativum*, *Nigella sativa*, *Apium graveolens*.

Kankayana Gutika is included (page 265) 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). Bangladesh National Formulary of Ayurvedic Medicine is compiled by the National Unani and Ayurvedic Formulary Committee and published by the Bangladesh Board of Unani and Ayurvedic Systems of Medicine, 75/B Indira Road, Dhaka-1215 under the authority vested in the Board vide section 13(j) of the Bangladesh Unani and Ayurvedic practitioners Ordinance, 1983 [3, 4].

Ayurvedic medicine uses a variety of products and practices. The safety profile of these drugs has not been fully investigated. It is also not clear, whether these preparations might interact with other drugs or diagnostic tests. Therefore the present study was carried out to evaluate the efficacy and safety of KKY in experimental animals.

MATERIALS AND METHODS

Drugs, Chemicals and Reagents: For the toxicological study, Kankayana Gutika (KKY) was collected from Sri Kundeswari Aushadhalaya Limited, Chittagong. Ketamine injection was purchased from ACI Limited, Bangladesh. All other reagents, assay kits and chemicals used in this work were purchased from Human GmbH, Wiesbaden, Germany.

Experimental Animals: Six to eight-week old male Sprague-Dawley rats 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 70-80 g. The animals were housed in a well-ventilated clean experimental animal house under constant environmental and adequate nutritional conditions throughout the period of the experiment. 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, Department of Pharmacy, Jahangirnagar University.

Experimental Design

Acute toxicity study: The acute oral toxicity test was performed following the guidelines of Organization for Economic Co-operation and Development (OECD) for testing of chemicals with minor modifications (OECD Guideline 425) [5]. Sixteen male mice (30-40 g body weight) were divided into four groups of four animals each. Different doses (1000 mg/kg, 2000 mg/kg, 3000 mg/kg and 4000 mg/kg) of experimental drug (KKY) were administered by stomach tube. The dose was divided into two fractions and given within 12 hours. Then all the experimental animals were observed for mortality and clinical signs of toxicity (general behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes and changes in skin and fur texture) at 1, 2, 3 and 4 hours and thereafter once a day for the next three days following KKY administration.

Chronic toxicity studies: Prior to the experiment, rats were randomly divided into 2 groups of 8 animals each. One group was treated with KKY 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 46 days. For all the pharmacological studies the drugs were administered per oral route at a dose of 400 mg/kg body weight [6]. After acclimatization, Ayurvedic medicinal preparation was administered to the rats by intra-gastric syringe between the 10 am to 12 am daily throughout the study period. All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals. The experiment animals were marked carefully on the tail which helped to identify a particular animal. By using identification mark, responses were noted separately for a particular period prior to and after the administration [7].

Overall Body Weight Analysis: Careful monitoring of body weights of rats of both groups were performed throughout the 46 days drug administration period. Body weights were recorded at regular intervals (2-3 days) until the treatment period was completed. All rats were kept under close observation throughout the experimental period. Statistical analysis of the initial and final growth rates were performed. The growth rate, expressed as percent increment in the body weight. The growth rate of the treatment group was compared with that of the Control group.

Organ Toxicity Study: At the end of the 46 days treatment period, the animals were fasted for 18 hours and also twenty-four hours after the last administration. Ketamine (500 mg/kg i.p.) was administered for the purpose of anesthesia [8]. Rats of both KKY and Control groups were sacrificed after the completion of the 46-day period and examined macroscopically for external lesions. Necropsy was performed to examine gross pathological lesions of various internal organs. Specific organs of interest

were then detached and preserved in 13% formalin and sent for the evaluation of histological anomalies. The tissues thus subjected to histo-pathological evaluation are: Heart, kidney, lungs, liver, spleen, thymus, stomach, caecum, pancreas, adrenal glands, urinary bladder, reproductive organs, which include testis, seminal vesicles, prostate gland and epididymis in case of males and ovaries, fallopian tube and uterus in case of females. Organs like heart, lungs, liver and spleen, portions of these tissues were excised and preserved for histological examination. The remaining portions were dried for determination of water content.

Relative weight of organ= $\frac{AOW}{BW} \times 100$
AOW= Absolute organ weight
BW= Body weight
Water content in tissue= $\frac{OW_1 - OD}{OW_1 - OF} \times 100$
OW_1 = organ wet weight
OD = organ dry weight
OF = organ foil weight

Statistical Analysis: The data were analyzed using independent sample *t*-test with the help of SPSS (Statistical Package for Social Science) Statistics 11.5 package (SPSS Inc., Chicago Ill). All values are expressed as mean ± SEM (Standard Error of the Mean) and $p<0.05$, $p<0.01$, $p<0.001$ was taken as the level of significant.

RESULTS

Acute toxicity study: The drug (KKY) administered up to a high dose of 4000 mg/kg produced no mortality. Thus the LD_{50} value was found to be greater than 4000 mg/kg body weight. According to the OECD test guideline 425 when there is information in support of low or non-toxicity and immortality nature of the test material, then the limit test at the highest starting dose level (4000 mg/kg body weight) was conducted. There were no mortality and toxicity signs observed at 4000 mg/kg body weight. Therefore, it can be concluded that KKY when administered at single dose is non-toxic and can be used safely in oral formulations.

Chronic growth study

Effect of KKY ***on Overall Body Weight*****:** The total treatment period was of 46 days. From 16th day onwards of the total treatment period of 46 days, the body weights of the KKY treated rats were found to be lower than the control group rats. It would be interesting to note here that in the initial 13 days the KKY treated rats were on the contrary having higher body weights, when compared with the control animals. None of these initial increase followed by decrease in body weights of the KKY treated rats were statistically significantly different from their corresponding body weights of the control animal.

Effect of KKY ***on Organ Toxicity Study:*** There is an ($p=0.208$, 4.10% decrease) decrease in the relative percent weight of the male rat heart, the decrease though not significant yet it was prominent. There is an increase in the relative percent weight of the male rat kidney ($p=0.258$, 4.57% increase), rat lungs ($p=0.164$, 11.22% increase), rat spleen ($p=0.076$, 16.52% increase) and rat testis ($p=0.348$, 64.64% increase), the increase though not significant yet it was prominent. There is a statistically very highly significant ($p=0.001$, 25.4 % decrease) decrease in the relative percent weight of the male rat liver. There is also a statistically significant ($p=0.026$, 35.8% decrease) decrease in the relative percent weight of the male rat thymus.

Effect of KKY ***on Tissue Hydration Index:*** There is an increase in the organ water content of the male rat heart ($p=0.400$, 0.83 % increase), rat kidney ($p=0.053$, 2.59% increase) and rat spleen ($p=0.140$, 5.69% increase), the increase though not significant yet it was prominent. There is a decrease in the organ water content of the male rat lungs ($p=0.358$, 1.41 % decrease), rat liver ($p=0.432$, 0.69% decrease) and rat testis ($p=0.347$, 2.25% decrease), the decrease though not significant yet it was prominent.

DISCUSSION

The evaluation of organ weights is fundamental to many biological studies. This is particularly true in the field of toxicological drug testing. To eliminate the well-known deviations found in absolute organ weights, the ratio of organ-to-body weight (in percent) is often used, whereas other reference parameters are sometimes preferred, brain or heart weight. But a survey of the relevant literature reveals equally wide deviations in studies of relative organ weights [9-11].

Overall Body Weight: The body weight should be measured at least once a week. Generally, in chronic studies, body weight is measured weekly for the first 13 weeks, and then monthly thereafter. A weekly measurement is recommended because rapid body weight loss is one of the most sensitive clinical indicators of test article-effect during the in life phase of the study and may reflect declining health, and may be prodromal to death. Rapid body weight loss may be due to decreased feed and/or water consumption, disease, dental maladies, or specific

toxic effects. Severe, rapid body weight loss can also prompt the removal of an animal from a study. Importantly, loss of body weight can impact the interpretation of organ weight data at the conclusion of a study [12]. In our study, from 16th day onwards of the total treatment period of 46 days, the body weights of the KKY treated rats were found to be lower than the control group rats.

Effect of KKY ***on Organ Toxicity Study:*** Dose-related increases in liver weight are commonly observed in repeat-dose toxicity studies performed in rodents. The causes of liver weight changes are diverse. One documented aged-related change in both humans and laboratory rodents is a decline in liver volume [13]. Here we found significantly decrease of liver weight to the KKY treated rats.

It can be difficult to make a clear distinction in routine toxicity studies from the thymic weight loss and here we found thymus weight decrease to the KKY treated rats but the result is not significant. Atrophy produced by agents with immune modulating properties from those compounds that produce similar morphological changes as a result of a generalized, high dose stress response. Indeed thymic involution, decreased responsiveness to Concanavalin A and lowered NK cell activity can be produced by a generalized stress response in a manner similar to that produced by the administration of glucocorticosteroids [14]. The dose-response relationship however is of some help in deciding whether thymic atrophy is a direct result of immunosuppression or a non-specific result of stress. Powerful immunosuppressive drugs such as cyclophosphamide produce thymic effects in a dose-related manner, with thymic weight loss and atrophy occurring at essentially non-toxic doses. By contrast, atrophy resulting from stress is usually limited to high doses where there is other clear evidence of stress-related phenomena such as general clinical depression, weight loss or other overt evidence of intoxication [15].

Effect of KKY ***on Tissue Hydration Index:*** The water content of tissues is essential to know in the development of physiologically based pharmacokinetic modeling [16] and in the interpretation of drug tissue distribution data [17, 18]. Changes in tissue water content can be also used to evaluate alterations in tissue physiology which are associated with an increase in tissue weight, such as the development of tissue edema [19-21]. In our study we found that KKY did not cause any significant increase or decrease in percent water content of any organs.

CONCLUSION

From the above experiment it can be concluded that KKY should not be administered chronically at a higher dose as it decreases percent relative weight of rat liver and thymus expressively. Further studies should be done by reducing the administered dose.

ACKNOWLEDGMENT

The authors are thankful to Focused Research on Ayurvedic Medicine and Education (F.R.A.M.E) Laboratory, Department of Pharmacy and all faculty members and the technical staffs of the Department of Pharmacy, Jahangirnagar University for their kind co-operation. We would express our special thanks to Mr. Shafiqul Islam for ensuring a constant supply of animals followed by proper maintenance and care of these animals during all throughout the experimental period.

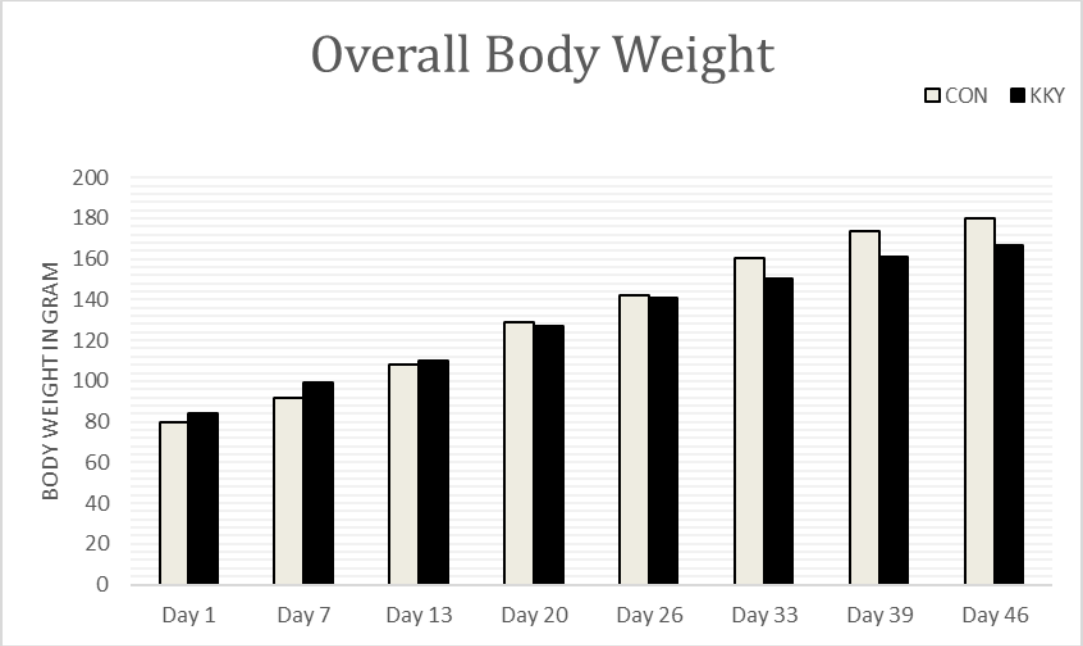

Figure 1: The effect KKY (400 mg/kg) on the body weights (g) of Spraque-Dawley rats with the time of treatment. Independent sample t-test was performed to analyze this weight variation in different days. All values are expressed as mean ± SEM and $p<0.05$, $p<0.01$, $p<0.001$ were taken as the level of significant

Table1: Name of the ingredients/herbs used in the preparation of Kankayana Gutika (KKY)

Name of ingredients	Plant part	Botanical name	Amount
1. Sati	Zadoary (root)	*Hedychium spicatum / Curcuma zeodaria*	48 g.
2. Puskara mula	root	*Inula racemosa*	48 g.
3. Danti	root	*Baliospermum montanum*	48 g.
4. Citraka	root	*Plumbago zeylanica*	48 g.
5. Adhaki	seed	*pigeon pea (Cajanus cajan)*	48 g.
6. Srngavera	rhizome	*Zingiber officinalis*	48 g.
7. Vaca	rhizome	*Acorus calamus*	48 g
8. Trivrta	root	*Operculina turpethum*	48 g.
9. Hingu (Exd.)		*Asa foetida*	144 g.
10. Yava ksara	Kshara of Barley	*Hordeum vulgare*	96 g.
11. Amlavetasa	Fruit	*Rheum emodi Wall.*	96 g.
12. Yamani	Fruit	*Hyoscyamus niger*	12 g.
13. Ajaji (jiraka)	Fruit	*Cuminum cyminum*	12 g.
14. Marica	Fruit	*Piper nigrum*	12 g.
15. Dhanyaka	Fruit	*Coriandrum sativum*	12 g.
16. Upakunci	Fruit	*Nigella sativa*	24 g.
17. Ajamoda	Fruit	*Trachyspermum ammi*	24 g.
18. Matulunga rasa		*Citrus medica*	Q. S.

Table 2: The effect of Kankayana Gutika (KKY) (400 mg/kg) on the relative organ weights of male rats

Parameters	Control	KKY	p value	% increase/decrease
Heart	0.2660±0.0077	0.2551±0.0026	0.208	4 .10 % decrease
Lung	0.5097±0.0331	0.5669±0.0225	0.164	11.22 % increase
Liver	3.6821±0.0820	2.7468±0.1028	0.001	25.4 % decrease
Kidney	0.3105±0.0040	0.3246±0.0112	0.258	4.57 % increase
Spleen	0.2566±0.0139	0.2990±0.0172	0.076	16.52 % increase
Thymus	0.1710±0.0098	0.1372±0.0157	0.026	35.80 % decrease
Testis	0.8063±0.0322	0.8176±0.0210	0.348	64.64 % increase

Independent sample t-test was performed to analyze this data set. All values are expressed as mean ± SEM and $p<0.05$, $p<0.01$, $p<0.001$ were taken as the level of significant

Table 3: The effect of Kankayana Gutika (KKY) (400 mg/kg) on various tissue hydration indices of male rats.

Parameters	Control	KKY	p value	% increase/decrease
Heart	76.9572±0.4223	77.5936±0.5859	0.4	0.83 % increase
Lung	79.9083±0.8359	78.7827±0.8437	0.358	1.41 % decrease
Liver	71.6569±0.2285	71.1660±0.5390	0.432	0.69 %decrease
Kidney	75.0393±0.8106	76.9811±0.5008	0.053	2.59 % increase
Spleen	71.0766±2.6650	75.1216±0.6508	0.14	5.69 % increase
Testis	75.4244±2.3823	73.7244±2.5323	0.347	2.2539 % decrease

Independent sample t-test was performed to analyze this data set. All values are expressed as mean ± SEM and $p<0.05$, $p<0.01$, $p<0.001$ were taken as the level of significant

REFERENCE

1. Agnivesha, Charaka samhita. Commentary: Vidyotini by Kasinatha Sastri and Gorakha Natha Chaturvedi, Sutrasthan Volume-I, chapter 30, verse no 26. Chaukhambha Bharati Academy Varanasi, India: 2011, pp. 259.
2. Sushruta, Sushruta samhita, Commentary: Ayurveda Tatva Sandeepika by Ambikadutta Shastri, Sutrasthan Volume I, chapter 01, verse no 22. Chaukhambha Sanskrit Sansthan Varanasi, India: 2010.
3. Anonymous. 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). National Unani and Ayurvedic Formulary Committee Bangladesh Board of Unani and Ayurvedic Systems of Medicine, 38, Bangabandhu Avenue, Dhaka-1000. (Second ed.), Dhaka, Bangladesh: 2011a.
4. Anonymous. 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). National Unani and Ayurvedic Formulary Committee Bangladesh Board of Unani and Ayurvedic Systems of Medicine, 38, Bangabandhu Avenue, Dhaka, Bangladesh: 2011b.
5. OECD Guideline (425) for the testing of chemicals. Guidance document on acute oral toxicity, Environmental Health and Safety Monograph Series on Testing and Assessment: 2008, pp. 1-27.
6. Gad S. C. Intl J Tox, 1988; 7(2): 127-38.
7. Stevens K. R. and Gallo M. A. Practical consideration in the conduct of chronic toxicity studies, *Principles and Methods of Toxicology,* 2nd edn. Chap. VIII: 1989.
8. Ringler H. and Dabich L. Hematology and clinical biochemistry. In: The Laboratory Rat Biology and Disease [Baker HL ed]. American College of Laboratory Animal Medicine Series Academic Press: 1979.
9. Webster SH, Liljegren EJ and Zimmer DJ. Amer. J. Anat, 1947; 81: 477-513.
10. Benitz KF, Morask RM and Cummings JR. Lab. Invest, 1961; 10: 936-46.
11. Ber A. Endokrinologie, 1972; 59: 187-96.
12. Adams, ET and Crabbs TA. Basic Approaches in Anatomic Toxicologic Pathology. Chapter 6 of Haschek and Rousseaux's Handbook of Toxicologic Pathology. Ed W.M. Haschek, C.G. Rousseaux and M.A. Wallig. 3rd revised ed., Elsevier Science: 2013, pp. 158-9.
13. Schmucker DL. Experimental Gerontology, 2005; 40: 650-9.

14. Monjan AA and Collector MI. Science, 1977; 196: 307-8.
15. Smialowicz RJ, Luebke RW, Riddle MM et al. Journal of Toxicology and Environmental Health, 1985; 15: 561-74.
16. Blakey GE. Tissue kinetics for a series of barbiturates: PhD Thesis. Department of Pharmacy, University of Manchester, Manchester, U. K: 1995.
17. Kato Y, Hirate J, Sakaguchi K, Ueno M, Horikoshi I. J Pharmacobiodyn, 1987; 10(9) :470-7
18. Khaiafallah N, Jusko WJ. J Pharm Sci, 1984; 73: 362-6.
19. Kanaiwa H, Kuchiwaki H, Inao S, Sugita K. Sue Netlrol, 1995; 44: 172-9.
20. Wang XD, Deng XM, Haraldsen P, Andersson R, Ihse I. Scan J Gastroenterol, 1995; 30:1129-36.
21. Heatherington AC. A physiological based pharmacokinetic model for drug distribution. PhD Thesis. Department of Pharmacy, University of Manchester, Manchester, U.K: 1994.