AYU

Access this article online
Website: www.ayujournal.org
DOI: 10.4103/0974-8520.123138
Quick Response Code:

Pharmacological Study

Acute toxicity study of *Vasaguduchyadi Kwatha*: A compound Ayurvedic formulation

Kalpu N. Kotecha, B. K. Ashok[1], Vinay J. Shukla[2], Pradipkumar Prajapati[3], B. Ravishankar[4]

Lecturer and Head, Department of Pharmacology, Institute of Ayurvedic Pharmaceutical Sciences, Gujarat Ayurved University, Jamnagar, Gujarat, [1]Drug Discovery Team, Research and Development, The Himalaya Drug Company, Makali, Bangalore , [2]Head, Pharmaceutical Chemistry Laboratory, [3]Professor and Head, Department of Rasa Shastra and Bhaishjya Kalpana, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, [4]Director, Reseach and Development, SDM Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi, Karnataka, India

Abstract

Vasaguduchyadi Kwatha is a compound Ayurvedic formulation, indicated for the treatment of liver diseases, especially for *Kamala* (jaundice) and *Panduroga* (anemia). However, till date, no safety profile of this formulation has been reported; hence, in the present study, freshly prepared and market samples of *Vasaguduchyadi Kwatha* were evaluated for acute toxicity. Acute toxicity test was evaluated as per OECD 425 guidelines with 5 000 mg/kg as limit test in Wistar strain albino rats. Test formulations were administered to overnight fasted animals and parameters like body weight, behavioral changes, and mortality were assessed for 14 days. Hematological and biochemical parameters were assessed on 14th day. Results showed no significant changes in terms of behavioral changes, mortality, and body weight. Both the samples did not affect any of the hematological parameters. However, increase in blood urea level was observed. This study shows that both the samples of *Vasaguduchyadi Kwatha* are relatively safe up to the dose of 5 g/kg. However, further chronic toxicity evaluation is necessary to establish its safety profile on chronic administration.

Key words: Acute toxicity, hepatoprotective, *Kamala*, *Vasaguduchyadi Kwatha*

Introduction

Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects.[1] In absence of a reliable liver protective drug in modern medicine, there are number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders.[2] However, only a small proportion of hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their safety and efficacy.[3] *Vasaguduchyadi Kwatha* is a compound Ayurvedic formulation explained in Astangahridaya[4] for the treatment of liver diseases, especially for *Kamala* (jaundice) and *Panduroga* (anemia). This formulation is extensively used by physicians to treat various liver diseases, especially in alcoholic liver disease. However, till date, no safety profile of this formulation is available. Many physicians use market samples, while some opt for freshly prepared formulation. Hence, in the present study, both fresh and market samples of *Vasaguduchyadi Kwatha* were selected for acute toxicity study.

Address for correspondence: Dr. Kalpu N. Kotecha, Lecturer and Head, Department of Pharmacology, Institute of Ayurvedic Pharmaceutical Sciences, A. K. Jamal Bldg, Gujarat Ayurved University, Jamnagar, Gujarat, India.
E-mail: k_kalpu@rediffmail.com

Materials and Methods

Test formulation

The plant materials [Table 1] of the test formulation were collected from pharmacy department and adjacent area of Jamnagar city after careful botanical identifications by referring to various botanical floras and with the help of pharmacognosist of the institute. These samples were converted to coarse powder (sieve no. 44) form and from the powder samples *Kwatha* (decoction) was prepared freshly according to the classical method[5] just prior to administration to the animals. In brief, 16 parts deionized water and one part drug which were boiled on low flame till 1/4th part was remaining. This was filtered and allowed to cool before administration. The prepared *Kwatha* contained 25 g of solid material in 100 ml. The rat weighing 200 g received 4 ml of *Kwatha* (1 g/200 g or 5 g/kg). The market sample of *Vasaguduchyadi Kwatha* was procured from market.

Animals

Female Wistar strain albino rats weighing 150 to 220 g were obtained from animal house attached to Pharmacology laboratory. Five animals in each group were housed in separate cage made up of poly-propylene with stainless steel top grill. The dry wheat (post hulled) waste was used as bedding material and was changed every morning. The animals were acclimatized for seven days before commencement of the experiment in standard laboratory conditions, 12 ± 01 hour day and night rhythm, maintained at 25 ± 3°C and 40 to 60% humidity. Animals were fed with Amrut brand rat pellet feed supplied by Pranav Agro Mills Pvt. Limited. For their drinking purpose, tap water *ad libitum* was used. Protocol used in this study for the use of animals was approved by the institutional animal ethics committee -Approval number; (IAEC – 04/08-10/PhD/02).

Acute oral toxicity study

Acute oral toxicity study for both freshly prepared sample as well as market samples were carried out following OECD guideline 425 (modified, adopted March 23, 2006) with 5 000 mg/kg as limit test. Food, but not water, was withheld for overnight before the experiment and further 2 hours after administration of test drug. The animals were observed continuously for 6 hours after the dosing for behavioral changes if found any. The careful cage side observation was done without disturbing the animal attention and at the end of every hour the animals were individually exposed to open arena for recording the behavioral changes like increased or decreased motor activity, convulsions, Straub's reactions, muscle spasm, muscle relaxation, salivation, diarrhea, hypoactivity, passivity, relaxation, ataxia, narcosis, etc., Furthermore, all the animals were observed for mortality during the entire period of the study. The body weight of each animal was recorded just prior to dosing on day one and 14th day. On 14th day, blood was collected by puncturing supra-orbital plexus by capillary tubes under ether anesthesia for estimation of hematological and biochemical parameters. To estimate hematological parameters, 0.08 ml blood was mixed with 0.02 ml of Ethylene Diamine Tetraacetic Acid-EDTA (33.33 mg/ml) and fed to the auto analyzer (Sismes KX-21, Trans Asia). The parameters measured were as follows: Total WBC, neutrophils percentage, lymphocyte percentage, eosinophils percentage, monocyte percentage, hemoglobin content, packed cell volume (PCV), total RBC, platelet count, mean corpuscular volume, mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

For estimation of biochemical parameters, serum was separated from collected blood and requisite quantity of serum was fed to the auto analyzer (Fully automated Biochemical Random Access Analyzer, BS-200; Lilac Medicare Pvt. Ltd., Mumbai) which was automatically drawn into the instrument. Biochemical parameters measured were blood sugar,[6] serum cholesterol,[7] serum triglyceride,[8] blood urea,[9] serum creatinine,[10] serum glutamic pyruvic transaminase,[11] serum glutamic oxaloacetic transaminase,[12] serum total protein,[13] serum albumin and serum globulin[14], serum alkaline phosphatase,[15] total bilirubin,[16] direct bilirubin,[17] uric acid,[18]and serum calcium.[19]

Statistical analysis

The results were presented as Mean ± SEM for five rats in each group. Statistical comparisons were performed by unpaired student's *t* test and One-Way ANOVA for all the groups with the level of significance set at $P < 0.05$.

Results

In the acute toxicity study no mortality was observed in treated rats and no toxic effect was observed throughout the 14 days study period at a dose of 5 000 mg/kg in both the samples of *Vasaguduchyadi Kwatha*. There were no changes observed in normal gross behavior of animals in both the treated groups.

The changes in body weight of the treated and control rats have been shown in Table 2. A normal progressive weight gain was observed in control group. In fresh sample of *Vasaguduchyadi Kwatha* administered group, marginal gain was observed, whereas in market sample administered group, significant increase in body weight was observed.

None of the hematological parameters was affected to significant extent in both the samples of *Vasaguduchyadi Kwatha* in comparison to control group [Table 3]. Among the 15 serum biochemical parameters, only one parameter was affected to significant extent [Table 4] and there was significant increase in blood urea level in both of the sample administered groups.

Discussion

The drugs intended to be used therapeutically should be subjected to toxicity evaluation before they are considered safe for use in the human beings. This is important because incomplete knowledge about the toxicity profile

Table 1: Formulation composition of *Vasaguduchyadi Kwatha* per liter

Drugs	Quantity used in equal proportion	Latin name	Part used
Vasa	31.25g	*Adhatoda vasica* Nees	Root
Guduchi	31.25g	*Tinospora cordifolia* (Willd.) Miers	Stem
Amalaki	31.25g	*Emblica officinalis* Gaertn	Peri carp
Haritaki	31.25g	*Terminalia chebula* Retz	Peri carp
Bibhitaka	31.25g	*Terminalia bellerica* Roxb	Peri carp
Chirayita	31.25g	*Swertia chirayita* (Roxb.) Karsten	Whole plant
Katuki	31.25g	*Picrochiza kurroa* Royle	Rhizome
Nimba	31.25g	*Azadirachta indica* A. Juss	Stem bark

16 times (4 l) of water was added and reduced to 1/4th (1l) of initial volume

Table 2: Effect on body weight

Groups	Initial body weight	Final body weight	Actual change
Control	196.80±7.73	208.80±7.39	12.00±1.09
Fresh *Kwatha*	204.00±13.25	212.40±11.62	08.40±4.07
Market *Kwatha*	172.00±11.57	199.60±09.62	27.60±6.49*

Data=Mean±SEM, *$P<0.05$ unpaired *t* test

Table 3: Effect on hematological parameters

Parameters	Control	Fresh *Kwatha*	Market *Kwatha*
WBC (cells/cumm)	7633.33±718.64	7740.00±1264.75	7040.00±713.16
Neutrophil %	17.00±1.92	25.60±6.23	16.20±1.65
Lymphocyte %	78.67±2.08	69.60±6.03	78.80±1.65
Eosinophil %	2.33±0.21	2.40±0.24	2.80±0.20
Monocyte %	2.00±0.00	2.40±0.24	2.20±0.20
Hemoglobin (g/dl)	14.93±0.42	13.54±1.68	16.08±1.08
PCV %	46.77±1.56	48.78±3.15	41.32±5.58
RBC (10^6/Cu mm)	8.17±0.26	7.16±1.01	8.47±0.52
Platelet (10^3/Cu mm)	1025.50±72.66	1153.20±112.73	1175.80±176.72
MCV (fl)	57.12±0.52	58.14±0.94	57.56±0.44
MCH (pg)	18.30±0.17	19.26±0.65	18.48±0.36
MCHC (g/dl)	32.01±0.18	33.12±0.67	32.90±0.30

Data=Mean±SEM. MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration

Table 4: Effect on serum biochemical parameters

Parameters	Control	Fresh *Kwatha*	Market *Kwatha*
Blood sugar (mg/dl)	100.80±03.60	114.50±06.91	102.00±07.08
Serum cholesterol (mg/dl)	66.50±6.99	57.60±2.29	61.00±11.48
Serum triglyceride (mg/dl)	85.66±5.70	82.00±13.48	95.00±15.12
Blood urea (mg/dl)	63.20±03.38	109.50±03.57#	102.50±03.79#
Serum creatinine (mg/dl)	0.56±0.033	0.62±0.037	0.64±0.024
SGPT activity (IU/l)	41.00±1.98	37.80±6.33	49.40±2.46*
SGOT activity (IU/l)	151.16±12.47	160.60±23.21	165.60±14.51
Total protein (g/dl)	7.23±0.23	7.38±0.20	7.46±0.33
Albumin (g/dl)	4.76±0.53	4.34±0.081	3.80±0.12
Globulin (g/dl)	3.06±0.26	3.04±0.19	3.66±0.29
A:G ratio	1.40±0.13	1.46±0.087	1.08±0.086
Alkaline phosphatase (IU/l)	177.50±24.46	149.20±27.99	129.60±12.31
Total bilirubin (mg/dl)	0.38±0.040	0.28±0.037	0.340±0.04
Direct bilirubin (mg/dl)	0.21±0.079	0.10±0.00	0.120±0.02
Serum uric acid (mg/dl)	0.91±0.19	1.04±0.06	1.08±0.06

Data=Mean±SEM, #$P<0.05$ One Way ANOVA, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase

of a putative drug will entail certain amount of risk to the recipient.[20] As explained in introductory part, the classical formulation *Vasaguduchyadi Kwatha* is extensively used clinically to treat various liver diseases; however, its acute toxicity profile is not reported till date. Hence, in the present study, acute toxicity of *Vasaguduchyadi Kwatha* has been evaluated.

Change in body weight is an important factor to monitor the health of an animal. Frequent Loss of body weight is the first indicator of the onset of an adverse effect and the dose, at which body weight loss is by 10% or more is considered to be a toxic dose, irrespective of whether or not it is accompanied by any other changes. In the present study, in test drug administered groups like control group, gain in body weight was observed and the magnitude of body weight gain was comparatively high in market sample of *Vasaguduchyadi Kwatha* administered group. Furthermore, at 5 000 mg/kg, sample did not produce any observable toxic effects during entire duration of study and all animals survived 14 days of observation. Both the samples did not affect any of the hematological parameters studied, while only the matter of concern in this study which can be taken as toxic effect is significant increase of blood urea; however, reason behind this change is a matter of further research.

Conclusion

From the observations recorded in acute toxicity studies for behavioral changes, hematological and biochemical parameters, body weight changes, and mortality, it is clear that both the samples of *Vasaguduchyadi Kwatha* were relatively safe. However, further chronic toxicity profiles are necessary to establish its safety profile on chronic administration.

References

1. Guntupalli M. Hepatoprotective effects of rubiadin, a major constituent of *Rubiacordifolia* Linn. J Ethnopharmacol 2006;103:484-90.
2. Chatterjee TK. Medicinal plants with hepatoprotective properties. In: Herbal Options. 3rd ed. Calcutta: Books and Allied (P) Ltd; 2000. p. 135.
3. Subramonium A, Pushpangadan P. Development of phytomedicines for liver diseases. Indian J Pharmacol 1999;31:166-75.

4. Vagbhata's. Astangahrdaya, translated by Prof. Srikantha Murthy. Chikitsa Sthana, Panduroga Chikitsa, 16/13 Krinshandas Ayurveda Series: 27. Varanasi: Krinshadas Academy; 2000. p. 449.
5. Sharangadhara Samhita, Jiwanprada hindi commentary, by Dr. Smt. Shailja Srivastava, Madhyam Khanda 9/3-5. 3rd ed. Varanasi: Chaukhambha Orientalia; 2003. p. 215.
6. Pennock CA, Murphy D, Sellers J, Longdon KJ. A comparison auto analyzer methods for the estimation of glucose in blood. Clin Chim Acta 1973; 48:193-201.
7. Roeschlau P, Bernt E, Gruber WA. Enzymatic determination of total cholesterol in serum. J Clin Chem Clin Biochem 1974; 12:226.
8. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem 1982; 2:2077-80.
9. Talke H., Shubert G E.: Enzymatic urea determinationin the blood and serum in the Warburg *optic*al test. Klin Wschr 1965;42:174.
10. Slot C. Plasma creatinine determination. A new and specific Jaffe reaction method. Scand J Clin Lab Invest 1965;17:381-7.
11. Burtis CA, Ashwood ER, editors. Tietz textbook of Clinical Chemistry. 3rded. Philadelphia, P.A.: Moss D.W., Henderson A.R.; 1999. p. 652.
12. Tietz NW, editor. Clinical guide to laboratory tests. 3rd ed. Philadelphia, PA: WB Saunders; 1995. p. 76.
13. Tietz NW, editor. Text book of Clinical Chemistry. Philadelphia, PA: W.B. Saunders; 1986. p. 579.
14. Doumas BT, Arends RL, Pinto PC. In standard methods of clinical chemistry. Vol. 7. Chicago: Academic Press; 1972. p. 175-89.
15. Wilkinson JH, Boutwell JH, Winsten S. Evaluation of a new system for kinetic measurement of serum alkaline phosphatase. Clin Chem 1969;15:487-95.
16. Pearlman PC, Lee RT. Detection and measurement of total bilirubin in serum with use of surfactants as solubilising agents. Clin Chem 1974;20:447-53.
17. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of clinical chemistry. 3rd ed. Philadelphia, PA: WB Saunders; 1999. p. 1136.
18. Kabasakalian P, Kalliney S, Wescott A. Determination of uric acid in serum, with use of uricase and tribromophenol-amino anti pyrine chromogen. Clin Chem 1973;19:522-4.
19. Tietz NW, editor. Textbook of clinical chemistry. Philadelphia, PA: W. B. Saunders; 1986.p. 1350.
20. Lipnick RL, Cotruvo JA, Hill RN, Bruce RD, Stitzel KA, Walker AP, *et al.* Comparison of the up-and-down, conventional LD50 and fixed dose acute toxicity procedures. Food Chem Toxicol 1995;33:223-31.

हिन्दी सारांश

वासागुडुच्यादि क्वाथ की तीव्र विषाक्तता का अध्ययन

कल्पु एन. कोटेचा, अशोक बी. के., विनय जे. शुक्ला, प्रदीप कुमार प्रजापति, रविशंकर बी.

वासागुडुच्यादी क्वाथ, यकृतरोगों, विशेषरूप से कमला रोग(पीलिया) और पान्डु रोग (एनीमिया) के उपचार के लिए अष्टांगहृदय में दर्शाया गया है । इस निरूपण का उपयोग व्यापक रूप से विभिन्न यकृत रोगों के लिए चिकित्सकों द्वारा किया जाता है । मद्यपान जनित यकृत विकारों में आज तक कोई सुरक्षा प्रोफाइल रिपोर्ट नहीं है । प्रस्तुत शोधपत्र में तुरन्त तैयार किया गया वासागुडुच्यादी क्वाथ और बाजार में उपलब्ध वासागुडुच्यादी क्वाथ के नमूनो की तीव्र विषाक्तता मूल्यांकन किया गया है । तीव्र विषाकता का मूल्यांकन ओई.सी.डी. ४२५ गाईडलाईन के दिशा निर्देशो के अनुसार – ५००० मि.ग्रा./कि.ग्रा. लिमिट में विस्टारएलबीनो चूहों पर कीया गया । टेस्ट निरूपण रातभर प्रशासित (कास्टेड) चूहों को दिया गया और शरीर भार, व्यवहारपरिवर्तन और मृत्युदर का १४ दिन तक अध्ययन किया गया । रक्तसंबंधी और जैवरासायनिक मानको का आंकलन १४ वे दिन कीया गया । अध्ययन दर्शाता है कि चूहों के व्यवहार, मृत्युदर या शरीरभार में कोई महत्वपूर्ण परिवर्तन नहीं पाया गया । रक्तसंबंधी मानको पर भी कोई महत्वपूर्ण परिवर्तन नहीं पाया गया । जैव रासायनिक मानकों में रक्तयूरिया स्तर में वृध्धि के अलावा और कोई महत्वपूर्ण परिवर्तन नहीं पाया गया । अध्ययन दर्शाता है की वासागुडुच्यादी क्वाथ के दोनों निरूपण अपेक्षाकृत सुरक्षित है लेकिन चिरकालिन प्रभाव के लिए आगे दीर्घ विषाक्तता मूल्यांकन करना जरूरी है ।