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A COMPARATIVE ACUTE TOXICITY STUDY OF SELF PREPARED AND MARKET SAMPLE OF *TRIBHUVANAKIRTI RASA*

Dubey Nidhi[1], Prafulla[2], Solanki Veer Bahadur[3]

[1,3]PG Scholar, [2]Lecturer,

Department of Agadtantra avam Vidhivaidyak Rani Dullaiya Smriti Ayurved PG College & Hospital, Bhopal,Madhya Pradesh, India

ABSTRACT

Tribhuvanakirti Rasa is a popular *Ayurvedic* formulation used widely in conditions like-all types of fever, spleen, liver diseases and said to improve digestion power. As this preparation contains many ingredients which are toxic in nature like *Vatsanabha* and *Hingula*. If it is not properly purified than it may cause toxic effects. In the present era due to the excess of commercialization many pharmacies produces substandard drugs for gaining more money which are having low quality, less therapeutic effect and may cause toxicity. There is world-wide demand of scientifically proved non-toxic Ayurvedic drugs.

Here the study is planned to access the best satisfactory results between available market sample and purified self-prepared sample to evaluate its acute toxicity in wistar strain albino rats. It was observed that at the dose of 90mg/kg body wt., at 4 times higher than the therapeutically equivalent dose, there was no mortality observed in acute toxicity study on different parameters studied.

Keywords:*Tribhuvanakirti Rasa, Hingula, Vatsanabha, Tankana, Shodhana*, Aconitine.

INTRODUCTION

Ayurvedic products can be made either of herbs only or a combination of herbs, metals and minerals. Some of these products can be harmful if used improperly or without the direction of a trained practitioner. *Ayurvedic* products have the potential to be toxic. Herbo-mineral preparations are believed to be fast acting and disease specific. Non purified heavy metals have been known for their toxicity. The possible heavy metal related toxicity arising from the use of herbo- mineral preparation is the subject of interest.

Tribhuvanakirti Rasa[1] is one of the most popular OTC product used liberally by *Vaidyas* and lay public alike. Rampant use has led to complaints in form of reporting adverse drug reactions. Strict implementation of Pharmaco-vigilance in recent era has brought this formulation in limelight. As this herbo-mineral preparation contains many ingredients which are toxic in nature like *Vatsanabha* and *Hingula*. These poisonous drugs are used in the medicinal preparations, after the *Shodhana*[2] procedure as mentioned in *Ayurveda*. *Shodhana* makes the materials adaptable to the body tissue like *Shodhita Vatsanabha* (aconite detoxified in cow's urine) is converted into cardiac stimulant, whereas crude *Vatsanabha* is claimed to be cardiac depressant.[3] Reported adverse events of *Tribhuvanakirti Rasa* have signs and symptoms like bradycardia, hypotension, palpita-

tions, perspiration etc. These symptoms are due to aconitine, a potent alkaloid present in *Vatsanabha*, one of the ingredients of such formulation. If *Shuddha Vatsanabha* is used as an ingredient, it should not produce lethal symptoms. But, recent developments in reporting of ADRs have created a lot of concern in this matter. In the present era due to the excess of commercialization many pharmacies produces substandard drugs for gaining more money. Which are having low quality, less therapeutic effect and may cause toxicity. The main reason behind this is proper *Shodhana* of all drugs needs more effort, time and money. There is world-wide demand of scientifically proved non-toxic Ayurvedic drugs. So for the acceptance of our drugs in modern society as well as to give a more clear explanation to the concept, pharmacological tests should be conducted. Here the study is planned to access assess the possible adverse effects if any between available market sample and purified self-prepared sample by carrying out acute toxicity study on wistar strain albino rats.

MATERIALS AND METHODS-

Collection of ingredients: Raw sample of *Hingula, Vatsanabha, Shunthi, Marich, Pippali, Pippalimoola* and *Tankan* were collected from Bhopal. Authentification of all raw ingredients was done at Shreeji Laboratory Indore.

Test drugs:

Test drug 1: Self-prepared Sample of *Tribhuvanakirti Rasa-* The method of preparation was as described in *Rasamratam*, Chapter 9: 80-801/2. Authentic herbal ingredients *Hingula*, *Vatsanabha*, *Shunthi*, *Maricha*, *Pippali*, *Tankana* and *Pippali moola* were used. *Hingula*[4], *Tankan*[5] and *Vatsanabha*[6] were purified as per Ayurvedic Formulary of India. Fine powders of all crude drugs were mixed thoroughly and successive *Bhavanas* were given one by one with *Tulsipatra swarasa*, *Ardrak swarasa*, *Dhaturapatra swarasa* and *Nirgundi patra swarasa*. After the completion of *Bhavana* the prepared mass was rolled in to *Vatis*. The pharmaceutical procedures were carried out in the pharmacy section of Rani Dullaiya Smriti PG Ayurveda College, Bhopal.

Test drug 2: Market Sample of *Tribhuvanakirti Rasa-* The market sample of *Tribhuvanakirti Rasa* was selected randomly. Market sample have premium quality and GMP certified. Because of medical ethics the brand name cannot be disclose here. For the further quality assessment standardization was done.

Standardizations of Drug – Standardization of both the test drugs was done at Shreeji laboratory Indore.

Animals:

Wistar and Charles Foster strain albino rats (*Rattus novergicus*) of either sex were used for the study. The animals were obtained from the animal house attached to Pharmacology laboratory of Shreeji lab Indore. The animals were exposed to natural day and night cycles under ideal laboratory conditions in term of ambient temperature (22+2 ^{0}C) and humidity (50-60%). The experiment was carried out in accordance with the direction of the Institutional Animal ethics committee (IAEC) after obtaining its permission (Approval number IAEAV; 01/14).

Dose fixation and schedule:

The dose calculation was done on the basis of body surface area ratio using the table of Paget and Barnes[7].

Acute toxicity study:

In acute toxicity study the drug was given in three dose levels, i.e. TED (Therapeutic equivalent dose) – 23mg/kg, TED×2 and

TED×4 i.e. up to 90mg/kg for higher dose level. This is to confirm with the concept of the dose limit test as suggested by OECD guidelines. Forty two animals were taken in 7 divided groups and each group comprising 6 animals.**(Table 1)**

Table 1: Grouping for acute toxicity study-

S.No.	Grouping	No. of rats	Drug administered	Dose/200gm body wt.	
01	Control group	6	Distilled water	2ml	
02	Trial group I(TKR-SP*)	6	Self-prepared *Tribhuvanakirti Rasa*	TED	4.5mg
		6		TED×2	9mg
		6		TED×4	18mg
03	Trial group II(TKR-MS**)	6	Market sample of *Tribhuvanakirti Rasa*	TED	4.5mg
		6		TED×2	9mg
		6		TED×4	18mg

*TKR-SP: Self prepared *Tribhuvanakirti Rasa*

**TKR-MS:Market Sample of *Tribhuvanakirti Rasa*

The test formulation was suspended in distilled water and administered orally with help of suitable of gastric catheter sleeved on to a syringe.

METHOD OF STUDY: It refers to recording of adverse signs and symptoms after the administration of single dose of drug at several levels higher than the therapeutically equivalent dose.

Charles Foster strain albino rats weighing between 173g ± 30g were used for evaluation of acute toxicity test. Acute oral toxicity study for both TKR-SP and TKR-MS samples were carried out following OECD guideline 401.

Examination of physical and behavioural changes: The animals were observed continuously for 6 hours after the dosing. 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 behavioural changes.

Observation: All the animals were observed at ½, 1, 2, 3, 4, 5, 6, 24 hours after dosing and there after daily once for mortality during the entire period of the study (14 days).

Body weight: The body weight of each animal was recorded just prior to dosing on day one, 7th and 14th day.

Terminal study: On 14th day all animals were kept for overnight fasting. Next day blood was collected by supra-orbital puncture with the help of micro capillary tubes under mild ether anaesthesia to collect blood to estimate haematological and serum biochemical parameters. Then the animals were sacrificed by over dose of ether anaesthesia. Further the rats were dissected and organs were separated and kept in normal saline (0.09%) carefully. All the organs were weighed with a monoplane balance and transferred immediately to a glass bottle containing 10% formalin. These samples were sent to the laboratory to carry out histopathological studies.

Parameters studied:

1. Ponderal changes:

The body weight of each animal was recorded just prior to dosing on day one, 7th and 14th day.

2. Haematological parameters:

The parameters measured were; haemoglobin percentage, WBC count, neutrophil percentage, lymphocyte percentage, eosinophil percentage, monocytes count, PCV,

RBC count, platelet count, MCV, MCH and MCHC.

3. Serum biochemical parameters:

Biochemical parameters like blood sugar, serum cholesterol, serum triglyceride, HDL cholesterol, blood urea, serum creatinine, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), serum total protein, serum alkaline phosphatase, total bilirubin, direct bilirubin, uric acid and serum calcium were estimated.

4. Histopathological studies:

Histopathology of important organs like brain, spleen, thymus, lymph node, heart, lungs, liver, intestine, kidney, testis, uterus and ovary has been carried out.

Statistical analysis: The data generated during the study were subjected to student's 't' test for unpaired data to assess the statistical significance. A 'P' value less than 0.05 is considered as statistically significant.

RESULTS AND DISCUSSION

Acute toxicity study: No behavioural changes (including cage side behaviour) were observed in both the treated groups. No mortality was observed in any of the group viz. TED, TED×2 and TED × 4.

In this study TKR was administered at three dose level viz., at therapeutic dose (23mg/kg) two times the equivalent to human therapeutic dose (46mg/kg) and four times the equivalent to human therapeutic dose (90 mg/kg).

In this study, the animals in both the test drug groups did not manifest any signs of toxicity up to 4 times (90mg/ kg) human therapeutic.

Haematological Study- Analysis of data related to haematological parameters after 14 days of test drugs administration revealed that TKR-SP and TKR-MS at a static dose was not shown any toxicity to any of haematological related parameters. Results are shown in table no.2. Table no.2 contains summary of the test drugs effect on haematological parameters. Total 12 parameters were studied.

In TKR-SP significant changes was observed in WBC, Neutrophils and Lymphocytes at all dose levels i.e. TED, TEDx2 and TEDx4. The observed change is the Significant decrease in the WBC, Neutrophil and Significant increase in Lymphocytes.

In TKR-MS significant changes was observed in WBC, Neutrophils, Lymphocytes, Monocytes, Platelets and MCHC at all dose levels i.e. TEDx2 and TEDx4. The observed change is the Significant decrease in the WBC, Neutrophil, Platelets and MCHC, while Significant increase in Lymphocytes and Monocytes.

Hence it can be stated that both the drugs are safe up to four fold to therapeutically equivalent dose in terms of haematological parameters.

Serological Study- Analysis of serological parameters shown in Table no.3, out of fourteen parameters, significant changes observed in following parameters;

There is Significant decrease of blood glucose level in therapeutic dose as well as in higher dose of both TKR-SP and TKR-MS sample. Hence it can be taken as pathological one.

Significant elevation in blood sugar was observed in TEDX4 dose of TKR-SP administered groups. This indicates that the test drugs may be mobilizing proglycaemic factors.

Significant increase of SGPT & SGOT in all the doses of TKR-SP and TKR-MS administered groups was observed in comparison to control group. In this parameter TKR-MS shows more significant increase than TKR-SP administered group.

Elevated blood urea and SGPT suggests inflammation of liver or heart. However histopathology of liver and heart did not show any pathological changes, hence the cause may by other than this. The reason behind this needs further elucidation.

Histo-Pathological Study- No changes were observed in either TKR-SP or TKR-MS.

So it was observed on overall basis that TKR-SP was safer than TKR-MS in term of haematological parameters, serum parameters, and histo-pathological parameters. And the reason for the safety of TKR-SP was the use of properly *Shodhit* and *Bhavita* contents with special precautions.

CONCLUSION

In case of acute toxicity, neither self- prepared *Tribhuvanakirti Rasa* nor market sample of *Tribhuvanakirti Rasa* show any toxic result at TEDX4 dose level. A number of biochemical parameters were assessed for the result of self-prepared *Tribhuvanakirti Rasa* and market sample of *Tribhuvanakirti Rasa*. In case of haematological and histopathological parameters none of them shown any changes but in serological parameters the self-prepared *Tribhuvanakirti Rasa* shows better results than market sample of *Tribhuvanakirti Rasa*. So on this basis, self-prepared *Tribhuvanakirti Rasa* is said to be more safer than market sample of *Tribhuvanakirti Rasa*. And the dose of *Tribhuvanakirti Rasa* can be increase up to four fold to the therapeutic dose as per the condition.

Table 2: Effect of TKR-SP and TKR-MS on various haematological parameters:

Parameter	NC	SP-TED	TEDx2	TEDx4	MS-TED	TEDx2	TEDx4
WBC (10^3/Cumm)	10405± 211.9	7803± 158.6	7773± 159.4	7743± 157.9	9305± 189.7	9355± 194.1	9406± 177.5
Neutro (%)	17.67 ± 0.33	12.33 ±0.21	12.00 ± 0.36	10.16± 0.60	15.83± 0.47	13.50 ± 0.42	12.66± 0.33
Lymp (%)	73.07± 0.37	80.43± 0.59	77.20± 0.46	74.30± 0.23	82.60± 0.52	80.28± 0.56	78.33± 0.42
Eosi (%)	2.83± 0.06	2.85± 0.07	3.18± 0.05	3.42± 0.03	3.01± 0.05	3.18± 0.05	3.38±0.03
Mono (%)	2.34± 0.02	2.34± 0.03	2.18± 0.02	1.68± 0.03	2.68± 0.02	3.03± 0.05	3.18± 0.02
TRBC(10^6/cu)	7.96± 0.42	7.86± 0.20	7.04± 0.08	6.20± 0.09	7.94± 0.23	8.06± 0.05	8.16± 0.06
Platelet (10^3/ l)	1114.23 ± 9.5	1117.58 ± 9.8	1152.44 ± 19.8	1235.1 ± 32.14	1066.86 ± 3.48	1002.7 ± 0.97	887.73± 11.65
MCHC(g/dl)	32.03± 0.09	31.90± 0.07	31.73± 0.14	31.83± 0.07	31.63± 0.01	31.63± 0.01	31.36± 0.03
Hb (g/dl)	14.58± 0.41	14.49± 0.39	14.60± 0.41	14.80± 0.18	14.44± 0.41	14.62± 0.42	14.80± 0.37
PCV (%)	45.41± 1.17	45.02± 1.11	46.24± 1.10	47.75± 0.70	45.77± 0.93	46.05± 1.14	47.08± 1.10

MCH(pg)	18.23± 0.45	18.27± 0.45	18.25± 0.13	18.37± 0.36	18.13± 0.43	17.81± 0.20	17.35± 0.11
MCV(fl)	56.90± 0.27	57.37± 0.21	57.20± 0.32	57.03± 0.03	57.23± 0.21	56.33± 0.23	55.43± 0.19

Table no.3: Effect of TKR-SP and TKR-MS on various Serological parameters:

Parameter	NC	SP-TED	TEDx2	TEDx4	MS-TED	TEDx2	TEDx4
Bld glu. (mg/dl)	111.0± 1.0	85.83 ± 2.0	100.45 ± 2.25	114.8± 1.34	104.51 ± 1.15	104.5± 1.12	104.51 ± 1.07
S.Chol (mg/dl)	58.5±1.6	60.3± 1.8	55.07± 0.99	50.07± 1.11	53.40± 1.24	54.05± 1.43	55.4± 1.15
S.Trigly (mg/dl)	94.3± 0.7	104.6 ± 1.9	107.07 ± 1.48	111.33± 1.00	107.35 ± 0.73	112.50 ± 0.51	117.99 ± 0.54
S.HDL (mg/dl)	43.3± 0.6	18.5± 0.6	27.8± 0.55	37.1± 0.77	36.3± 1.01	36.8± 0.90	37.3± 0.78
Bldurea (mg/dl)	51.6± 0.6	62.3± 0.7	72.5± 0.47	73.0± 0.54	65.3± 0.76	76± 0.58	86.6± 0.37
S.Crtn (mg/dl)	0.5± 0.0	0.5± 0.0	0.5± 0.01	0.5± 0.18	0.5± 0.01	0.58± 0.01	0.58± 0.16
T. protein (g/dl)	7.9± 0.2	7.6± 0.3	7.4± 0.19	7.3± 0.18	7.7± 0.27	7.80± 0.23	7.85± 0.29
SGPT (IU/L)	40.5± 1.4	48.3± 1.3	51.1± 0.22	59.8± 1.28	50.1± 1.46	57.00± 0.31	62.8± 0.7
SGOT (IU/L)	148.8± 0.5	150.8 ± 0.3	152.6± 0.53	154.3± 0.4	153.6± 1.04	155.2± 0.71	157.5± 0.6
S.Alk.Phos.(IU/L)	160.5± 0.5	167.5 ± 0.4	195± 0.46	202.6± 0.81	193± 0.64	197.8± 0.5	222.5± 0.8
S.Bili(T) (mg/dl)	0.40± 0.0	0.48± 0.0	0.50± 0.00	0.53± 0.00	0.47± 0.00	0.52± 0.00	0.56± 0.00
S.Bili(D) (mg/dl)	0.15± 0.0	0.12± 0.0	0.15± 0.00	0.18± 0.00	0.12± 0.00	0.17± 0.00	0.22± 0.00
S.Uricacid (mg/dl)	1.22± 0.0	0.93± 0.0	0.88± 0.00	0.91±0.00	0.85± 0.00	0.82± 0.00	0.71± 0.00
S. Cal.	8.50± 0.03	8.82± 0.0	7.8± 0.04	6.7± 0.00	8.35± 0.03	8.35± 0.04	8.35± 0.06

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CORRESPONDING AUTHOR

Dr.Dubey Nidhi

PG Scholar Department of Agadtantra avam Vidhivaidyak
Rani Dullaiya Smriti Ayurved PG College & Hospital,
Bhopal,Madhya Pradesh, India
Email: dubeydrnidhi@gmail.com

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