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RESEARCH ARTICLE

SAFETY AND TOXICOLOGICAL EVALUATION OF VRIKHAMLA HERBAL FORMULATION FOR WEIGHT MANAGEMENT

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ABSTRACT

Aim: The current study was designed to study the toxicological evaluation of Vrikshamla herbal formulation for weight management.

Material and method: The whole study also includes the standardization of selected herbal formulation with various parameters like Ash value, extractive value, moisture content, pH and phytochemical investigation. Herein we assessed the broad spectrum safety of Vrikshamla in a battery of in vitro and animal toxicological studies such as, acute oral toxicity studies (1000mg/kg and 2000 mg/kg orally of test sample) was conducted to determine the safe dose as per OECD-423 guideline. The primary skin and eye irritation tests on rabbits classified Vrikshamla drug according to Draize mathod, acute dermal toxicity study in mice.

Result: No sigh of toxicity was observed in all parameters except mildly irritating to the eye of rabbit.

Conclusion: These results, combined with the tolerability of Vrikshamla in the human clinical trials, demonstrate the broad spectrum safety of Vrikshamla.

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INTRODUCTION

Obesity has grown into a worldwide epidemic in recent year. Accumulating evidence indicates that obesity is a risk factor for other diseases such as type 2diabetes, cardiovascular diseases and certain cancer including colon cancer and breast cancer. In fact it is estimated that obesity may reduce life expectancy by 7 years age 40. Accordingly, the global socioeconomic burden for obesity and its related disorders is tremendous and is expected to continue increasing. (Saiyed Zainulabedin, 2015). Herbal medicines derived from plants. They are used as supplements to improve health, and may be used for other therapeutic purposes. Herbal products are available as tablets, capsules, powders, extracts, teas and so on. Herbal medicines are considered to be safe as it is natural, but in fact it can cause serious adverse effects and interaction with other drugs and supplements. Some of the advantage of herbal drugs are high low / minimum cost, fewer side effects, have large amount of use (Dugs.com). Herbal drugs imply knowledge and practice of herbal healing for the prevention, diagnosis, and elimination of physical, mental, or social imbalance. The costs for health care are rising at an alarming rate throughout the world. At the same time, the world market for phytopharmaceuticals is growing progressively.

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It is a common observation that people diagnosed with incurable chronic disease states such as diabetes, arthritis, and AIDS turned to herbal therapies for a sense of control and mental comfort from taking action (Kalyani Pathak, 2013). With the help of herbal drug, found any toxicological symptoms on mice and rabbits. The degree to which a substance (a toxin or poison) can harm humans or animals. Acute toxicity produce harmful effects in an organism through a single or short-term exposure. Chronic toxicity is the capability of a substance or mixture of substances to cause harmful effects over an unlimited period, usually upon repeated or continuous exposure, sometimes lasting for the entire life of the exposed organism (Fauci Anthony, 2008).

MATERIALS AND METHODS

Drugs and Chemicals

Vrikshamla Drug, methnol, iodine, potassium iodide, conc. sulphuric acid, chloroform, HCL, magnesium ribbon, acetic acid, NaOH, Hager's reagent, Wagner's reagent, Mayer's reagent, Dragendroff's reagent, gelatin, foam, froth, ferric chloride, starch, molish, phenolic compound, salkowski test.

Animals

Swice albino mice (28-35gm) and albino young rabbits (1-2 kg) were used in the present study. The animals were procured

from disease free small animal house. They were provided normal diet and tap water and were exposed to 12hr. Light and 12hr.dark cycle. The animal was acclimatized to the laboratory condition before experiments. Experimental protocol was approved by Institution (IPS-College of pharmacy) animal ethics committee. Care of animal were taken as per guidelines of the committee for the purpose of control and supervision of experiments on animal (CPCSEA), ministry of environment and forest government of India. The animals were kept in polypropylene cages under standard laboratory condition maintained at 27°c ±2°c temperature and 50 to 60% humidity.

Dose

1-2 tablets of *VD* twice a day with meal.
Dose of VD was calculated for mice in the present study following the OECD
Guideline number 423.

Extraction

Ten tablets from each formulation were taken randomly and weigh for the further evaluation. The tablets were crushed to powder form for extraction with suitable solvent system after examining the solubility property of the drugs. Distilled water solvent system was the preferable solvent system for the study as per the available literature. Simple maceration technique of extraction was followed to get the purified drug for further investigations. (Kokate, 2005).

1.Preliminary Phytochemical study
Determinationof ash value, Loss of drying,determination of extractive value, determination of P^H value.

2.Phytochemical Screening
Test For Alkaloids, Carbohydrate, Saponins, Tannins, Phenol, Starch, Steroids.

3.Pharmacological Investigation

Acute oral toxicity: Acute oral toxicity study was carried out to determine the safe dose by acute toxic class method of oral toxicity as per Organization for Economic Co-operation and Development (OECD)423 guideline (Anonymous-2001). The overnight fasted mice(n=3/group) were orally administered *VD* in limit dose of 1000mg/kg(group-1) and 2000mg/kg(group-2) and observed continuously for behavioral, neurological and autonomic profile for 2 hours and after period of 24,72 hours and thereafter up to 14 days for any lethality, moribund state or death.

No of animal per group=3/group

a. Group I administered with 1000mg/kg of test drug.
b. Group II administered with 2000mg/kg of test drug.

Primary eye irritation study in rabbit

- **Preparation of dose:** Take 1.633 mg of powdered drug according to the body wt. of rabbit and then solubilize in 10 ml triple distilled water. Then the solution was filtered from micro filter paper, and the filtered solution was then transferred into the vial, then that vial was kept in autoclave for sterilized at 60 degree centigrade for (15-20 min.), after this the vial was taken out from the autoclave.
- **Procedure:** One albino young rabbit was taken. The route of *VD* administration was direct conjunctival instillation, standard for assessment of local ocular irritative potential. Before the instillation, both eyes of each animal were examined for gross abnormalities according to the Draize scale for scoring eye lesions. Then 2 drops of prepared drug was then instilled into the conjunctival sac of the right (test) eye of rabbit by softly pulling the lower lid away from the eyeball. Following treatment, ocular irritation will be evaluate macroscopically using a high intensity white light in accordance with Draize (1944) at 1, 24, 48, and 72 h and daily from 4 to 10 days post-instillation. Individual eye irritation scores were recorded of rabbit.

Primary dermal irritation study in rabbits

Two albino young adult rabbits were taken. The route of *VD* administration was through a direct application of test substance to clipped intact skin. On the day before application, hair was removed by clipping the dorsal and the trunk area. On the day of dosing, but prior to application, the animals were examined for health and the skin abnormalities. To apply material, 2000mg/kg of test substance was moistened with water then applied to a small area of the clipped skin and covered with gauze patch. Access by the animal to the patch and ingestion or inhalation of *VD* was prevented.

Individual dose sites were scored according to the Draize scoring system (Saxena Rahul, 2007) at approximately 1, 24, 48, and 72 h after removal of *VD* patch. The classification of irritancy was obtained by adding the average erythema and edema scores for 1, 24, 48, and 72 h scoring intervals and dividing by the number of evaluation intervals. The resulting Primary Dermal Irritation Index (PDII) was classified according to the descriptive rating. Animals were also observed for signs of gross toxicity and behavioral changes at least once daily during the test period.

Acute dermal toxicity in mice

Four male mice were used in this test. Individual doses of the *VD* were calculated based on the initial body weights obtain prior to dosing at 2000 mg/kg of body weight (bw). On the day prior to application, the hair was removed by clipping the dorsal area and the trunk. After clipping and prior to application, the animals were examined for health, weighed (initial) and the skin checked for any abnormalities. *VD* was moistened with distilled water to achieve a dry paste. *VD* (2000 mg/kg BW) was then applied to a 2 in × 3 in, 4-ply gauze pad and placed on the dorsal area of the animal (approximately 10% of the body surface).

The gauze pad and the entire trunk of each animal were wrapped with 3-inch Durapore tape to avoid dislocation of the pad and to minimize loss of the test substance. The mice were then returned to their designated cages. The day of application was considered day 0 of the study. After 24 h of exposure to the test substance, the pads were removed and the test sites were gently cleaned of any residual test substance. The body weight of each animal was recorded prior to test substance application and again on days 7 and 14. Animals were observed for mortality, signs of gross toxicity, and behavioral

changes for several hours after application and at least once daily for 14 days. Observation included evaluation of skin and fur, eye, behavioral. Attention was paid to the occurrence of salivation and coma. (Saiyed Zainulabedin, 2015).

RESULTS

1. Standardization of Vrikshamla Drug

Table 1. Showing the values for various parameters for Standardization of VD

S.No.	Standardization Parameter	Value
1.	Ash analysis	
	• Ash content (Total ash)	28%
	• Acid insoluble ash	13.5%
	• Water soluble ash	12.6%
2.	Extractive value (Maceration process)	
	• Methanol soluble	0.75%
	• Water soluble	4.25%
3.	Moisture content (Loss on Drying)	3%
4.	pH (1% aqueous solution)	8.12

2. Preliminary phytochemical Screening

Table 2. Preliminary phytochemical screening of *VD*

S.No.	Phyto constituents	Phytochemical test	Result
1.	Starch	Iodine test	-
2.	Alkaloids	1.Hager's test	+
		2.Wagner's test	++
		3.Mayer's test	+
		4.Dragendorff's test	++
3.	Saponins	1.Froth test	-
		2.Foam test	-
4.	Carbohydrates	Molisch's test	+
5.	Terpenoids	Salkowski's test	++
6.	Tannins	1.Gelatin	+
		2.Ferric chloride test	-
7.	Phenols	Phenolic compound	-

(+) Positive, (++) Moderated Positive, (-) Negative

3. Pharmacological Investigation

3.1.Acute oral toxicity study

Table 3. Acute oral toxicity study of *VD* in mice (male) at various dosed

S.No.	Dose	Observation Period (24 hr)	Observation Period (14 days)
1.	1000 mg/kg	All animals survived	No sign of toxicity, normal diet and feeding.
2.	2000 mg/kg	All animals survived	No sign of toxicity, normal diet and feeding.

Table 4. Acute oral toxicity study [observation table for weight (gm)]

S.No.	Days	Group I/1000mg/kg			Group II/2000mg/kg		
		Anima I	Animal II	Animal III	Animal I	Animal II	Animal III
1	Ist Day	40.50	38.36	38.41	32.59	35.62	33.00
2	3rd Day	41.29	40.75	39.00	33.50	35.98	34.36
3	7th Day	41.67	42.89	40.45	31.00	36.88	34.52
4	14th Day	34.64	31.52	33.00	30.67	34.12	33.57

a.Number of animals (n=6)
b.Number of animals in group I = 3
c.Number of animals in group II = 3

3.2.Primary eye irritation study in rabbit

Table 5. Incidence of positive effects, severity and reversibility of ocular irritation in PEI study

Time postinstillation		Incidence of positive effects		
		Corneal Opacity	Iritis	Conjuctivitis
Hours	1	0	0	A3 , C2
	24	0	0	0
	48	0	0	0
	72	0	0	0
Days	4	0	0	0
	5	0	0	0
	6	0	0	0
	7	0	0	0
	8	0	0	0
	9	0	0	0
	10	0	0	0

a.Number of animals (n=1)
b.Maximum mean total score of 1 animals
For scoring see Draize Scale

Test side

Control side

Figure 1. Test and control side of rabbit eye

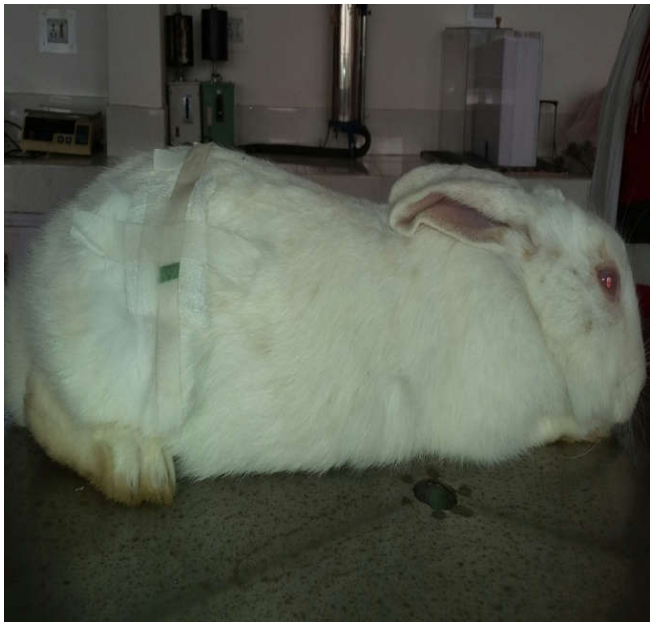
3.3.Primary dermal irritation study in rabbits

Table 6. Evaluation of reactions (Draize Methods) in PDI study

Hours / Score	Rabbit I		Rabbit II	
	Control	Test	Control	Test
1 Hrs.				
Erythema Score	0	0	0	0
Edema Score	0	0	0	0
24 Hrs.				
Erythema Score	0	0	0	0
Edema Score	0	0	0	0
48 Hrs.				
Erythema Score	0	0	0	0
Edema Score	0	0	0	0
72 Hrs.				
Erythema Score	0	0	0	0
Edema Score	0	0	0	0

a.Number of animals (n=2)
b.Test Side = Left
c.Control Side = Right

Test Side



Control Side

Figure 2. Test and control side of rabbit skin

3.4. Acute dermal toxicity study in mice

Table 7. Weight (mg) observation in acute dermal toxicity study

Days	Control Group		Test Group	
	Animal-I	Animal-II	Animal-I	Animal-II
0 Day	34.78	35.47	37.53	36.00
7 Day	33.65	35.12	38.90	36.73
14 Day	35.81	36.00	39.26	37.34

Table 8. General appearance and behavioural observations for control and treated group

Observation	Control Group		Test Group	
	Animal-I	Animal-II	Animal-I	Animal-II
1 Hrs.				
Skin and fur	Normal	Normal	Normal	Normal
Behavioural	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal
Coma	No	No	No	No
24 Hrs.				
Skin and fur	Normal	Normal	Normal	Normal
Behavioural	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal
Coma	No	No	No	No
48 Hrs.				
Skin and fur	Normal	Normal	Normal	Normal
Behavioural	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal
Coma	No	No	No	No
72 Hrs.				
Skin and fur	Normal	Normal	Normal	Normal
Behavioural	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal
Coma	No	No	No	No
7 Days				
Skin and fur	Normal	Normal	Normal	Normal
Behavioural	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal
Coma	No	No	No	No
14 Days				
Skin and fur	Normal	Normal	Normal	Normal
Behavioural	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal
Coma	No	No	No	No

a.Number of animals (n=4)
b.Test group (male) = 2
c.Control group (female) = 2

DISCUSSION

- The physiochemical parameter viz ash content, extractive value moisture content pH indicated that the formulation. *VD* intended for study was of inquisite pharmacopeial standard. Phytochemical analysis is very important in the evaluation of active biological component of plant. The phytoconstituents quantified in the present study exhibit great deal of medicinal importance specially alkaloidal compound is major roll play in toxicological activity quantitative estimation of *vrikshamla drug.*
- Acute oral toxicity study of the *VD* (1000mg/kg and 2000mg/kg orally) invealed that there was no toxicity to any nature, all animals survive during observation period.

The study was undertaken in view of the therapeutic use of *VD* ayurved a system of medicines for treatment of various disease like- weight loss, joint pain, treating

warm, emptying the bowel, severe diarrhea etc. The toxicological activity effect of ayurvedic formulation were investigated in present study.

- In the primary eye irritation study in rabbits, one hour after *VD* instillation, treated eyes exhibited conjuctivital discharge. The overall incidence and severity decreased with time and became zero on day 10. Based on these results, *VD* was classified as mildly irritating to the eye.
- In primary dermal irritation study in rabbits, there was no sign of dermal irritation, adverse pharmacological effects or abnormal behaviour. The primary dermal irritation assay using a single (2000mg/kg) dose of *VD* applied directly to the skin of rabbits for 24 hrs. did not cause any dermal irritation, thereby allowing *VD* to be classified as non-irritating to the skin.
- In acute dermal toxicity study in mice, after applying of *VD* there was general behavioral pattern of mice was shown and no toxic symptoms or mortality in any animals, which lived up to 14 days after the administration of the test compound at dose level of (2000 mg/kg) in acute dermal toxicity study. The behavioral patterns of animals were observed 1hr and followed by 14 days after the removal of patch and all animals were found normal and did not display any significant changes in behavior, skin effects, impairment in food intake and water consumption, postural abnormalities and hair loss.

Conclusion

The evaluate of Ayurvedic Formulation Vrikshamla on acute oral toxicity study, primary eye irritation study in rabbit, primary dermal irritation study in rabbits, acute dermal toxicity study did not show any toxicological sign. Body weight and estimated feed consumption were not affected. No mortility was observed and did not observe any gross lesions and histopathological changes in all treated group. Therefore, the test substance VD can be considered safe for human therapeutic use at the recommended therapeutic doses and regimen for long use. Hence it is practically non toxic.

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