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Toxicological and Pharmacokinetic Analysis by Insilico Bioinformatics Tools of an Ancient Medicine Compound Cantharidin Derived from Spanish fly

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Abstract

Background: Cantharidin, a vesicant released by spanish fly belongs to species beetles of zoological order Coleoptera. It has a long history in both folk and traditional medicine use for topical cantharidin to treat warts and molluscum and as an aphrodisiac. They are also known to cause poisoning by the presence of toxic principle.

Aims and Objectives: The aims of the study were to study possible drug likeness, pharmacokinetic and toxicological profiling of known toxicological active principle cantharidin by insilico analysis and prediction tools.

Materials & Methods: This study was investigated on web-based tools PubChem to extract the chemical structure, followed by authentication and validation with the chemical formula. The two-dimensional structures are further converted to three-dimensional (3D) structure with ChemSketch software; The structures are then screened for molecular properties with Simplified Molecular Input Line Entry System, followed by absorption, distribution, metabolism, elimination, and toxicity through SWISS ADME software web based tool. The reports are analyzed and predicted for drug likeness, pharmacokinetic and toxicity characters of cantharidin.

Results: The compounds screened cantharidin for drug likeness had a Log P score of >4.15, Bioavailability Score of 0.55, +ve blood brain barrier +ve Intestinal penetration and -ve to Cytochrome P450 enzyme induction and inhibition. The toxic hazard classification by Cramer resulted as HIGH –CLASS III with Skin Sensitization score of 0.861

Conclusion: The insilico analysis predicts cantharidin belongs to class III Toxic hazard classification but potential as drug like candidate for topical administration due to its high skin sensitivity and irritation ability as a vesicant in treatment of warts and molluscum.

Keywords: cantharidin, Toxicology, Spanish fly, bioinformatics

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Introduction

Cantharidin, commonly known as "Spanish fly," In asian continents, cantharidin topically was used historically for furuncles and piles, ulcers, venomous worms, and tuberculous scrofuloderma.[1,2] and for abdominal masses[3] and rabies, as well as an abortifacient4 it was used oral preparation.

It has been used in folklore as a sexual stimulant. Cantharidin poisoning is reported by cutaneous exposure, unintentional inoculation, and inadvertent ingestion of the beetle itself.

Blister beetles are plant-eating insects that exude a blistering agent. They can be found in the eastern United States, southern Europe, Africa, and Asia. When the beetle senses danger, it exudes cantharidin by filling its breathing tubes with air, closing its breathing pores, and building up body fluid pressure until fluid is pushed out through one or more leg joints[5].

Cantharidin is readily absorbed from all surfaces, including the skin and topically on application to the skin, redness and burning pain are produced which is followed by formation of vesicles.

Cantharidin on oral ingestion, there is burning sensation of mouth, throat and abdomen followed by nausea and vomiting of blood stained material, pain in abdomen, severe thirst, tenesmus and difficulty in swallowing and speech. Later, a dull pain is felt in the loins, desire to micturate, but urine is scanty and bloodstained. Priapism in males and abortion in pregnant females may occur. The patient becomes prostrated with convulsions and coma preceding death at a fatal dose of 15-30 mg of cantharidin or 1.5 g of powder with a fatal period of 24hr6.

Cantharidin in a collodion vehicle has been used by dermatologists as a treatment for molluscum contagiosum. The present study explores toxicological aspects and pharmacokinetic predictions of cantharidin using *insilico* bioinformatics tools.

Aims and Objectives

The aims of the study were to study possible drug likeness, pharmacokinetic and toxicological profiling of known active principle cantharidin by insilico analysis and prediction tools.

Materials and Methods

Hardware and Software The selected compounds molecular properties of chemical structure from Spanish fly active principle cantharidin are carried out in Hewlett Packard PC 32-b0390 in 2021 Model installed with windows 11 java enabled with updated plugins.

Data Set The chemical structures of cantharidin from Spanish fly with two-dimensional (2D) pictures were collected from accredited indexed published journals and other sources such as PubChem, Chembank, ChemPDB, and Asinex Ltd. After a detailed review, the structures are developed with ChemSketch, followed by PHASE software module was used to convert the 2D structures into three-dimensional (3D) structures[7].

Virtual Screening

Drug likeness, Pharmacokinetic and Toxicological Prediction

The 3D structures developed are explored virtually using online prediction software[8]. Results from insilico analysis of compounds on drug likeness, pharmacokinetics and toxicity are acquired in the virtual screening workflow protocol

Statistical Methods and Calculation

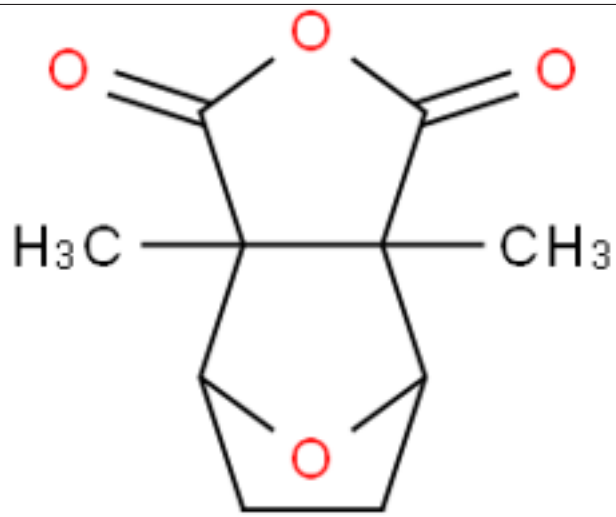
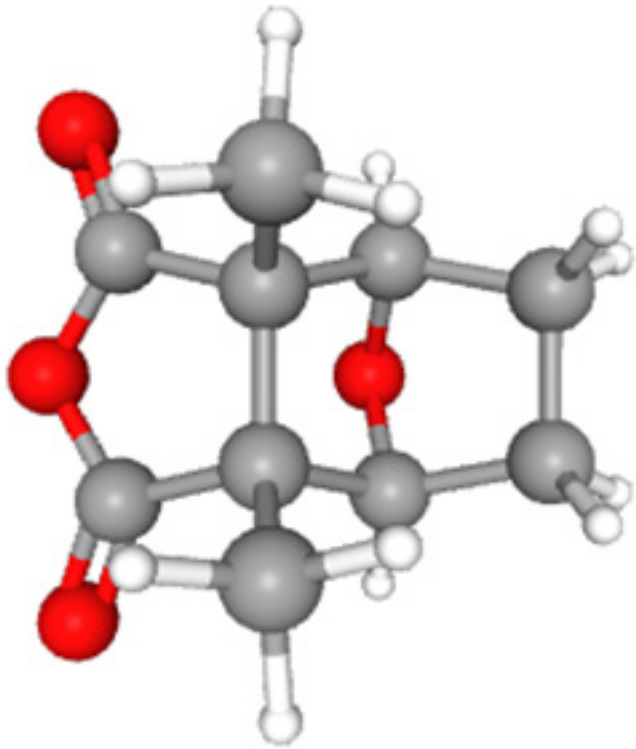
Interactive molecular properties calculator applet (MolSoft L.L.C. San Diego, CA, USA) is used for molecular volume and drug-likeness score. The study is done in the department of Forensic medicine, Pharmacology and the college digital library using online tools during January 2022 to March 2022. The study was self-funded conducted at SVIMS University, Tirupati, Andhra Pradesh, India. The study is considered under the category for exemption from institutional ethics committee approval as it does not involve animals and humans and done by insilico bioinformatics tools.

Results and Discussion

The present study is designed to study and analyze Toxicological properties of cantharidin followed by a prediction of pharmacokinetic parameters based on results obtained by bioinformatics experimental models. In Table 1 the 2D chemical structure of

cantharidin were retrieved from PubChem online compound database. The 2D structures converted to 3D structure by ChemSketch software[9]. The 3D structures are the processed with MOLSOFT L.L.C Software, and the molecular properties are predicted (Table 1).

Table 1: Spanish fly compounds 2D, 3D structure & Physiochemical properties

Sl. No	Characters	Cantharidin
1.	2D Structure	
2	3D Structure	
3	Chemical Formula	C10 H12 O4
4	Molecular weight	196.2 g/mole
5	Num. Hbond acceptors	4
6	Num. Hbond donors	0

H-bond-Hydrogen bond

The molecular weight was 196.2 g/mole with high Hydrogen bond acceptor site of 4 bonds and zero donor bonds. This combined with high gastrointestinal absorption and Log Kp value of -7.09cm/s and bioavailability score 0.55 is predicted as drug like candidate with zero violations as per Lipinski's rule[10]. Lipophilicity plays a significant role in drug discovery and compound design. The lipophilicity of an organic compound can be described by a partition coefficient, log P. (Table 2)

Table 2: ADME predicted profile of Spanish fly active compounds

Sl. No	Pharmacokinetic Parameters	cantharidin
1.	G.I Absorbtion	High
2	BBB	Yes
3	P-gp Substrate	No
4	CYP450 3A4 Inhibitor	No
	CYP450 3A4 Inducer	No
5	$\text{Log } K_P$	-7.09cm/s
6	Druglikeness	Yes; 0 violation: MLOGP>4.15
7	Bioavailability Score	0.55

MLOGP -Moriguchi octanol-water partition coefficient, log kp - Human skin permeability coefficients

Lethal Dose value is the amount of a solid or liquid material that it takes to kill 50% of test animals in one dose. It is also called the median lethal dose. The Toxicological prediction over lethal dose 50 values on various routes in mouse model revealed LD 50 value of 0.37 mg/kg/d in subcutaneous route with low reliability index and other routes predicted with borderline prediction reliability index as shown in Table 3A.

Table 3A: Toxicology prediction profile of Spanish fly active compounds

Sl. No	Species	LD50(mg/Kg/d)	Reliability Index
1.	Mouse Oral	770	0.4
2.	Mouse Intraperitoneal	4.42	0.46
3.	Mouse Intravenous	36.38	0.48
4.	Mouse Subcutaneous	0.37	0.28

Reliability index: <0.3 = Not reliable prediction quality; 0.3-0.5 = borderline prediction quality; 0.5-0.75 = moderate prediction quality; >0.75 = high prediction quality

The Cramer classification scheme is an guideline to predict and estimate the Threshold of Toxicological Concern (TTC) for a chemical substance based on its chemical structure. Cantharidin chemical structure is predicted with class III representing the most severe toxic hazard. The Table 3B results predict cantharidin with positive prediction to cause skin sensitization with a value of 0.861 and allergic contact dermatitis positive prediction. The predictions results revealed positive potential towards carcinogenicity and negative teratogenic potential

Table 3B: Toxicology prediction profile of Spanish fly active compounds

Sl. No	Toxicology Profile	Cantharidin
1.	Toxic hazard classification by Cramer	HIGH –CLASS III
2.	Skin Sensitization	0.861-POS-IN
3.	Allergic contact dermatitis(human & guinea Pig)	POS-IN
4.	Respiratory Sensitization	POS-OUT

Sl. No	Toxicology Profile	Cantharidin
5.	Teratogenic potential (human Model)	NEG-OUT
6.	Cancer Male & Female Mouse	POS-IN

POS-Positive, NEG-Negative, IN-Inside Applicability domain, OUT-Outside, Applicability Domain**.**

Cantharidin in a collodion vehicle has been used by dermatologists as a treatment for molluscum contagiosum and warts since the 1950s[11,12]. High skin sensitization and allergic dermatitis may suggest cantharidin possibly inhibits protein phosphatase activity resulting in endothelial permeability by elevated albumin flux and dysfunction of barrier[6]. The clinical effects can mostly be attributed to the irritative effects on the exposed organ systems. The secretions cause an urticarial dermatitis that is manifested several hours later by burns, blisters, or vesiculobullae. Symptoms may be immediate or delayed over several hours.

In addition to the local effects, predicted results as class III -Toxic hazard classification by Cramer (**Table-3B**) cantharidin can cause systemic toxicity with diaphoresis, tachycardia, hematuria, and oliguria from an extensive dermal exposure. When ingested, severe GI disturbances and hematuria can occur. Initially patient complaints may include burning of the oropharynx, dysphagia, abdominal cramping, vomiting, and hematemesis followed by lower GI tract symptoms of hematochezia and tenesmus. The aphrodisiac properties are related to cantharidin's ability to cause vascular engorgement and inflammation of the genitourinary tract leading to priapism and pelvic organ engorgement[13].

Conclusion

Cantharidin historically as ancient medicine used by asian continents as vesicant and used by dermatologists as a treatment for molluscum contagiosum and warts. Studies over cantharidin have exhibited vasoconstrictor activity and positive inotrope effect in guinea pig and human cardiac tissue in vitro[14,15]. Although the current study predicts cantharidin is too toxic belonging to class III toxic hazardous substance to administer systemically, it is possible to develop drug like candidate for its ionotropic effects on heart and topical application with safer derivatives as the druglikeness score is within applicability domain.

Conflict of Interest: None declared.

Ethical clearance: No Humans or animals involved in study.

Refernces

1. Wang GS Medical uses of mylabris in ancient China and recent studies. J Eth-nopharmacol. 1989;26:147-162.
2. Mawangtui Han Tomb Silk Book, Part 4, Beijing, China: Cultural Objects Pub-lishers; 1985.
3. Museum of the Gansu Province: Wu Wei Han Dynasty Medical Tablets II. Bei-jing, China Cultural Objects Publishers; 1957.
4. Cheng KC, Lee HM, Shum SF, Yip CP. A fatality due to the use of cantharides from Mylabris phalerata as an abortifacient. Med Sci Law. 1990; 30:336-340.
5. Narayan reddy K.S. and murthy O. The Essentials of Forensic Medicine and Toxicology. 34th ed. New Delhi: Jaypee brothers medical publishers; 2017.
6. Biswas Gautam. Review of Forensic Medicine and Toxicology 2nd ed. New Delhi: Jaypee brothers medical publishers; 2012.
7. Fan Y, Cheng M, Cheng Y. The reformation in our organic chemistry classes caused by the excellent software ACD/ chemsketch. J Guangzhou Univ 2002;6:12-32.
8. Daina, O. Michielin, V. Zoete. SwissADME: a free web tool to evaluat pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci. Rep. 2017; 7:42717. doi: 10.1038/srep42717.
9. Fan Y, Cheng M, Cheng Y. The reformation in our organic chemistry classes caused by the excellent software ACD/ chemsketch. J Guangzhou Univ 2002;6:12-32.
10. Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discov Today Technol. 2004 Dec;1(4):337-41. doi: 10.1016/j.ddtec.2004.11.007. PMID: 24981612.
11. Funt T Cantharidin treatment of molluscum contagiosum. Arch Dermatol. 1961; 83:186-187.

12. Epstein W, Kligman A. Treatment of warts with cantharidin. Arch Dermatol. 1958; 77:508-511.
13. Ellenhorn MJ. Ellenhorn's Medical Toxicology: Diagnosis and Treatment of Hu-man Poisoning. 2nd ed, Baltimore, Md: Williams & Wilking; 1997.
14. Neumann J, Herzig S, Boknik P, et. al. On the cardiac contractile, biochemical and electrophysiological effects if cantharidin, a phosphatase inhibitor. J Pharmacol Exp Ther. 2000 Jul;61(1):43-50.
15. Linck B, Boknik P, Knapp J, et. al. Effects of cantharidin on force of contraction and phosphatase activity in non failing and failing human hearts. Br J Pharmacol.1996;119:545-550.