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Acute Oral Toxicity Study of *Paris polyphylla* extract in Rats

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Abstract

Paris polyphylla Smith is regarded as "Wonder Herb" due to its vast therapeutic applications ranging from diarrhea to cancer. The cytotoxic effect of the rhizome extract on the healthy rats was monitored over a period of time. The extract proved non cytotoxic as animals gained weight and no signs of mortality, ill health or overt toxicity were observed. A brief period of dullness indicates a possible role of the extract in neuro-suppression. The cutoff median lethal dose (LD_{50}) of extract after single oral administration to female rats, observed over a period of 14 days is found to be greater than 5000 mg/Kg body weight. Thus it can be used in the treatment of neurological disorders associated with hyper active neurons. Moreover as the extract is cytotoxic to cancerous cells only it would prove good target for future studies on anti cancer drugs. The results of this study collectively specify that oral administration of *Paris polyphylla* is not connected with any toxicologically significant effects and the data could provide satisfactory preclinical evidence of safety to launch a clinical trial on a standardized formulation of the plant extracts.

Keywords: *Paris polyphylla*; Rhizome; Hydroalcoholic Extract; Cytotoxic.

Introduction

Paris polyphylla grows largely in the temperate regions and tropical areas of Europe and Asia. It is one of the important plants in traditional Indian and Chinese system of medicine. *Paris polyphylla* is regarded as "Wonder Herb" due to its vast therapeutic applications ranging from diarrhea to cancer. The plant has its ethno-vetinary use for fever, stomach disorders and shoulder wounds of oxen [1]. The rhizomes of *Paris polyphylla* have been used as anti-helminthic and vermifuge by tribes of Nepal [2-6]. The roots have diverse roles as pain reliever, anti-inflammatory, antipyretic, antispasmodic, antitussive, antileishmanial, antioxidant, antipyretic, depurative, sedative and spermicidal activities [1, 7-11]. The roots extract is used in the treatment of poisonous snake bites, boils and ulcers, diphtheria and epidemic Japanese B encephalitis [8]. The paste of the roots is used as an ointment to treat cuts and wounds [6]. The powered roots of this plant are used to treat diarrhea in Himalayan regions of India [12]. Rhizomes of *Paris polyphylla* are rich in saponin glycosides, which are being used as haemostatic agents and promoters for shrinkage of uterus in clinics. [13, 14]. Traditionally it is also used for anti microbial [15] and anti-cancer action [9, 16-19]. *Paris polyphylla* has also displayed anti-tyrosinse activity [20]. Thus effective for the treatment of dermatological disorders associated with melanin hyperpigmentation. The saponins of *Paris polyphylla* rhizome activate immunological reactions, including removal of foreign substance [21]. The anti-fungal activity of *Paris* saponin against *Cladosporium cladosporioides* and *Candida species* has also been confirmed [16, 22].

Phytochemical study showed that its main components, steroidal saponins possess a potential cyotoxicity against various tumor cell lines, such as CCRF leukemia cells, EC109 esophageal cancer cells, CaEs-17 cells, human promyelocytic leukemia HL-60 cells, human liver carcinoma HepG-2 cells, human gastric cancer BGC-823 cells, human colon adenocarcinoma LoVo cells human lung cancer A549 cells and SW-116 cells [17, 23-26]. Recently, polyphyllin D, a natural compound from *Paris polyphylla*, have been found with anti-neoplastic activity on ovarian cancer (OVCA) cell line proliferation and platinum sensitivity [27, 28].

In our previous studies [29], we have used hydrolcololic extract from rhizomes of *Paris polyphylla* to analyze its cytotoxic potential on human lung cancer A549 cells lines and concluded that it exhibits anticancer activity. In order to analyze the effect of the extract on normal cells also here, in the present article we are studying the cytotoxic effect of the extract on the healthy rats.

Material and Method

Material & Extraction

The rhizome of *Paris polyphylla* was procured from Patanjali Natural Coloroma and stored in ambient conditions for further study. The other solvents and chemicals were purchase from Sigma-Aldrich, India.

The rhizomes of the plant were dried in shade for about 3 weeks and ground using a mixer to a coarse powder. Using a soxhlet extraction method, the powder of dried rhizome was processed with petroleum ether (40-50ºC) for 18 h in order to remove fat and unwanted components. The treated powder was further processed with hydroalcoholic solution (60:40) by using same extraction procedure for 18h. The extract was evaporated to dryness in a

rotary flash evaporator at a temperature not exceeding 60 C, then stored in air tight container.

Approximately 200 mg of the extract was dissolved in 1 ml of distilled water and vortexed. The extract was completely soluble and a solution of 200 mg/ml was obtained.

Animals

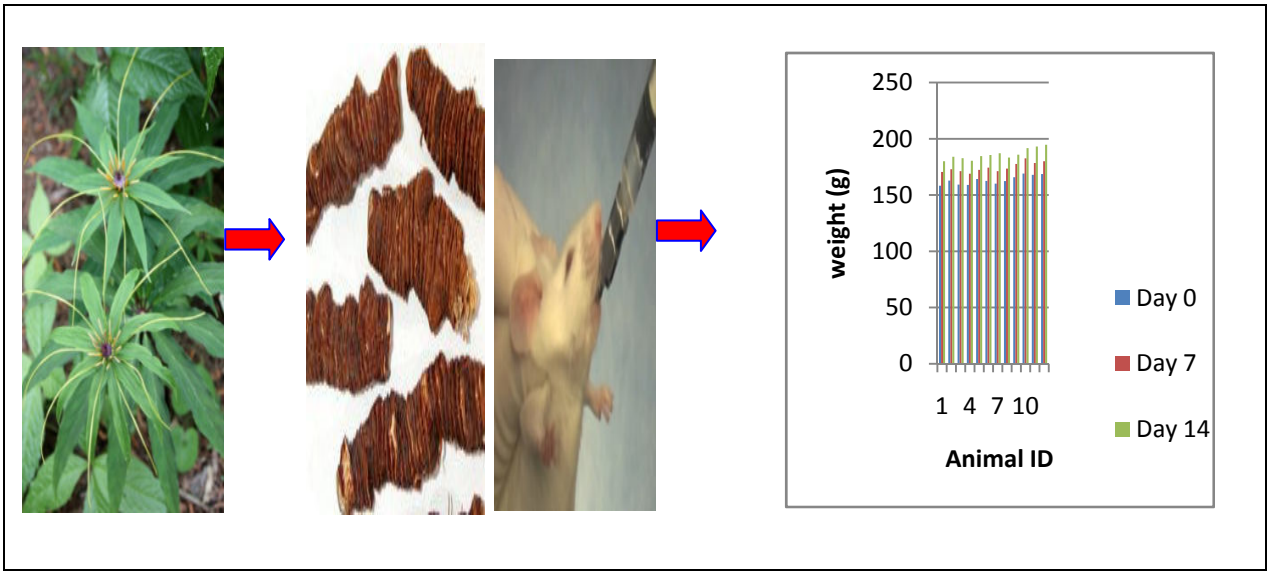
Female Wistar rats (8-12 weeks, weighing between 158-171 g) were used for the experiment. All animals were maintained under standard laboratory conditions, with a constant 12 h light/dark cycle and controlled temperature (22 ± 2 C) with access to drinking water and pellet diet (Lipton India Ltd.) *ad libitum*.

This study was performed in compliance with the current GLP requirements of National GLP Compliance Monitoring Authority (NGCMA), India and the OECD Principles of Good Laboratory Practice ENV/MC/CHEM(98)17 following all guidelines for testing of chemicals, number 423, "Acute Oral Toxicity-Acute Toxic Class Method."

Experimental protocol

The female Wistar rats were acclimatized for 6 days. A stepwise procedure with the use of 3 rats per step was used in the study to access the acute toxicity of the extract. The starting dose was 300 mg/Kg body weight. The animals received a single dose of the extract by oral administration after being fasted overnight but with free access to water. The food was provided again at approximately 3 h after the administration of extract. The extract was formulated in distilled water at concentration of 200 mg/ml and administered the dose volume of 10 ml/Kg. The animals were observed daily during the acclimatization period, mortality/viability and clinical signs were recorded. All animals were observed for clinical signs during first 30 min and at approx. 1, 2, 3 and 4 h after administration on test day 0 and once daily during test days 1-14. Mortality/viability was recorded during first 30 min and at approx 1, 2, 3 and 4 h after administration on test day 0 and twice daily during days 1-14 (at least once on day of sacrifice). Body weights were recorded on test day 0 (prior to administration), test days 7 and 14 of the experiment. All the animals were sacrificed at the end of 14 day observation period by CO_2 in euthanasia chamber and discarded after gross/ macroscopic pathological changes were observed and recorded.

Results and Discussion



All the animals dosed at 300 mg/Kg and 2000 mg/kg body weight showed dullness during first 15 min after administration after which they remained normal throughout the experimental period. No other signs of ill health or overt toxicity were observed **(Table 1)**. A brief period of dullness indicates a possible role of the extract in neuro-suppression as well.

Table 1-Effect of oral administration of *Paris polyphylla* extract on animal behaviour and mortality.

Dose (mg/Kg body weight)	Animal ID	0*day				Day 1 to 14	Mortality
		15 min	30 min	45 min	60 Min		
300	1	D	N	N	N	N	Zero
	2	D	N	N	N		
	3	D	N	N	N		
300	4	D	N	N	N		
	5	D	N	N	N		
	6	D	N	N	N		
2000	7	D	N	N	N		
	8	D	N	N	N		
	9	D	N	N	N		
2000	10	D	N	N	N		
	11	D	N	N	N		
	12	D	N	N	N		

N=Normal, D=Dullness

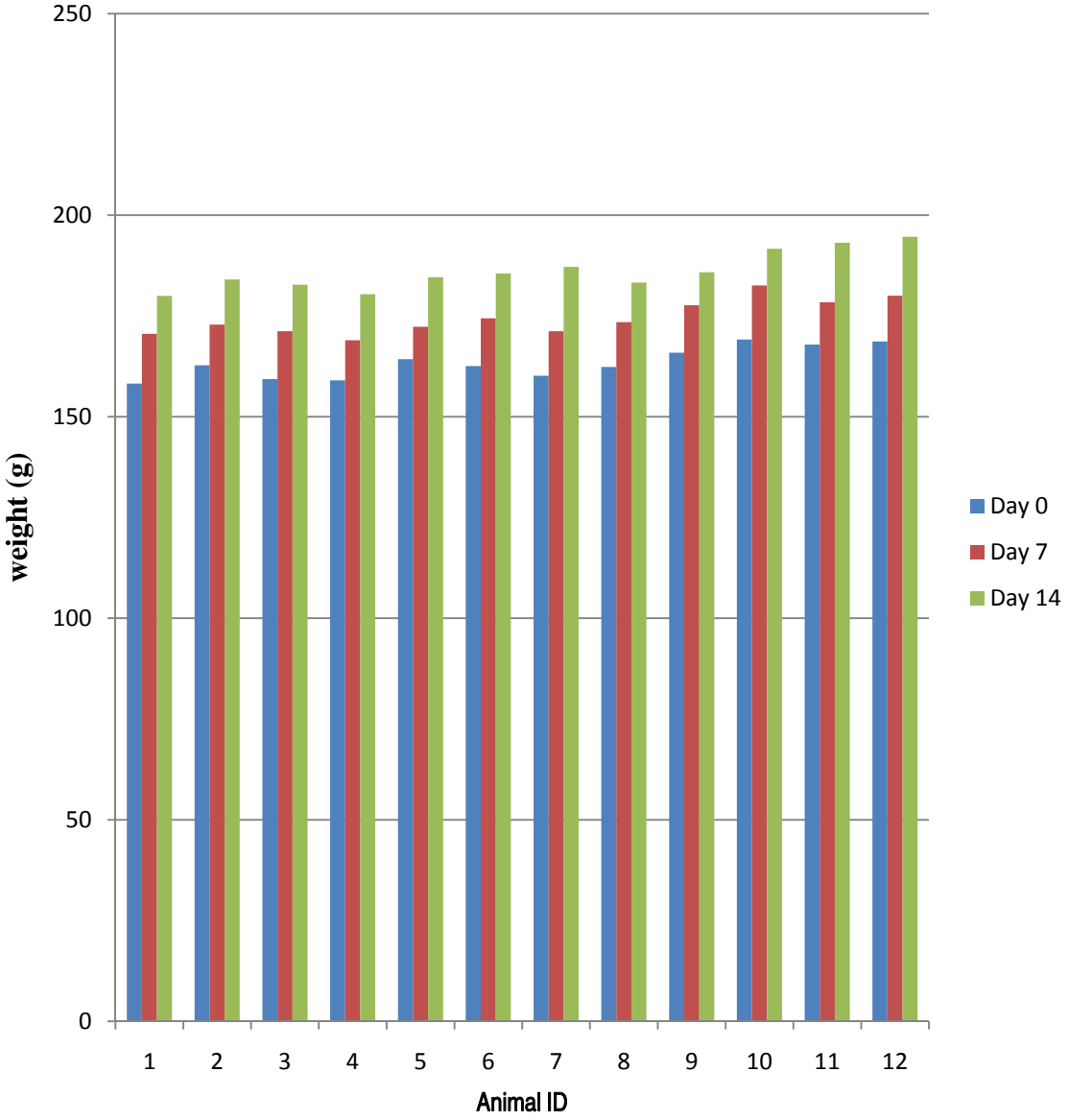
*Examinations were performed within the first 15 min and at approximately 30, 45, 60 min after treatment on test day 0

No gross lesion was observed in the rats on gross pathological examination. No mortality was observed in any animal after administration of the extract. All the animals had gained body weight by day 14 as compared to day 0 **(Table 2, Figure 1)**. The average weight gains observed in the animals are considered to be within the range of commonly recorded body weight in this strain.

Table 2-Individual Body Weight in Grams over a period of 14 days after oral administration of *Paris polyphylla* extract

Dose (mg/Kg body weight)	Animal ID	Day 0 (Weight in Grams)	Day 7 (Weight in Grams)	Day 14 (Weight in Grams)
300	1	158.21	170.56	179.98
	2	162.76	172.87	184.05
	3	159.34	171.23	182.77
300	4	159.04	168.98	180.39
	5	164.29	172.32	184.64
	6	162.56	174.40	185.52
2000	7	160.18	171.22	187.18
	8	162.34	173.45	183.29
	9	165.87	177.67	185.86
2000	10	169.14	182.55	191.68
	11	167.90	178.43	193.15
	12	168.65	180.00	194.66

Figure 1-Graphical presentation of Individual Body Weight in Grams over a period of 14 days after oral administration of *Paris polyphylla* extract



Based on the results, the extract from *Paris polyphylla*, falls under 'Category 5 or Unclassified' according to the Globally Harmonised System (GHS) for the classification of chemicals. The cutoff median lethal dose (LD_{50}) of extract after single oral administration to female rats, observed over a period of 14 days is found to be greater than 5000 mg/Kg body weight.

In our previous studies [29], we used hydrolcololic extract from rhizomes of *Paris polyphylla* on human lung cancer A549 cells lines to access its cytotoxic potential using MTT assay. More than 97 % increment in cell killing at a concentration of 500 µg/ml was recorded. The EC_{50} value of 52.34 µg/ml and 0.579 µg/ml was calculated for the extract and Doxorubicin, respectively. **(Figure 2a & 2b)**

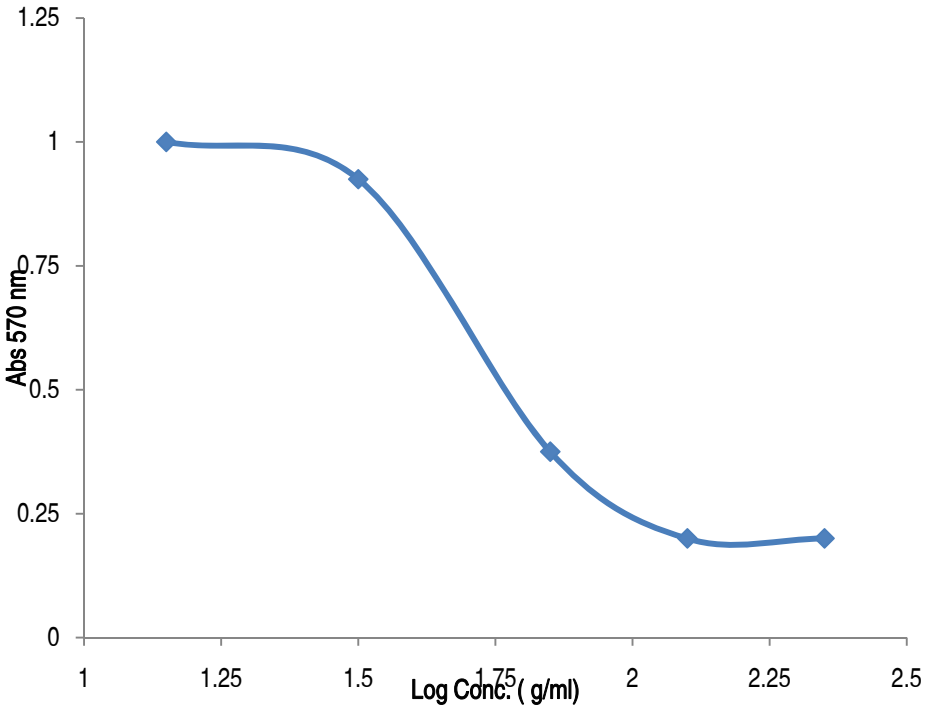


Figure 2 (a)- EC_{50} value of extract in µg/ml[29]

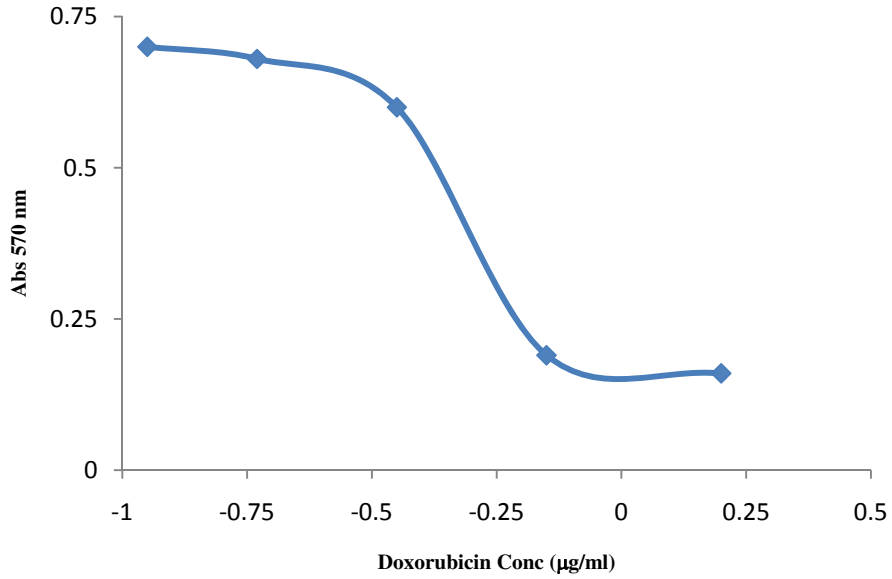


Figure 2 (b)-EC_{50} value of Doxorubicin in µg/ml[29]

Comparing the *in vitro* and *in vitro* results it becomes obvious that *Paris polyphylla* extract is toxic only to cancerous cells and does no harm to healthy cells. Thus this result is beneficial for future cancer research.

Conclusion

The cytotoxic effect of the extract on the healthy rats was monitored over a period of time. The extract proved non cytotoxic as animals gained weight and no signs of mortality, ill health or overt toxicity were observed. A brief period of dullness indicates a possible role of the extract in neuro-suppression. Thus it can be used in the treatment of neurological disorders associated with hyper active neurons. Moreover as the extract is cytotoxic to cancerous cells only it would prove good target for future studies on anti cancer drugs. To summarize, the results of this study collectively specify that oral administration of *Paris polyphylla* is not connected with any toxicologically significant effects and the data could provide satisfactory preclinical evidence of safety to launch a clinical trial on a standardized formulation of the plant extracts.

Conflict of Interest

All authors declare that they have no conflict of interest.

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