Acute Oral Toxicity of *Abelmoschus manihot* and *Wrightia tinctoria* in Mice

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ABSTRACT

Abelmoschus manihot and *Wrightia tinctoria*, belonging to the botanical family Malvaceae and Apocynaceae, have been traditionally used by the locals in India for treatment of various ailments. The current study reports the outcome of acute oral toxicity investigation of *Abelmoschus manihot* and *Wrightia tinctoria*, on ICR mice. No mortalities or evidence of adverse effects have been observed in ICR mice following acute oral administration at the highest dose of 2500 mg/ kg crude extracts of *Abelmoschus manihot* and *Wrightia tinctoria*. This is the first report on the acute oral toxicity of *Abelmoschus manihot* and *Wrightia tinctoria* and the findings of this study are in agreement with those of *in vitro* experiments and thus provide scientific validation on the use of the leaves of *Abelmoschus manihot* and *Wrightia tinctoria.*

Key words: Acute oral toxicity, Malvaceae, Apocynaceae, *Abelmoschus manihot, Wrightia tinctoria.*

INTRODUCTION

Medicinal herbs have always been used as traditional primary healthcare agents, especially in Asian countries. Over the last 20 years, rapid changes have been observed in the popular use of natural products from plant sources for maintenance of health and for alternative therapy, in Western countries.[1]

Abelmoschus manihot commonly known as "Jungli Bhindi" in **India** belong to botanical family Malvaceae, is a large annual erect hairy plant, 1.2-1.8 m. high. It is native to China, was introduced into India, near Calcutta and in coastal areas of Maharashtra. The mucilage contains polysaccharides and proteins.[2] The flower contains quercetin-3-robinoside, quercetin-3'-glucoside, hyperin, myrecetin and anthocyanins.[3] The different chromatographic methods have been developed on the flavones present in the plant.[4,5] The flowers are used in the treatment of chronic bronchitis and toothache. The ethanol extract of flower was screened for antiviral activity, and it was observed that the hyperoside shown significant anti HBV activity.[6] The flavones present in the plant showed preventive effect in the injury.[7] The leaves were tested on bone loss in ovarectomised rats and it was observed that it was able to prevent the ovariectomy-induced femoral osteopenia[8] **whereas the woody stem extracts possess analgesic activity.[9]**

Wrightia tinctoria commonly known as Dhudh Kodi in India *b*elong to the botanical family Apocynaceae**,**[10] is a small deciduous tree, generally up to 1.8 m tall and often under 60 cm girth, sometimes up to 7.5 m high, distributed all over India. Four uncommon sterols, desmosterol, clerosterol, 24-methylene-25-methylcholesterol and 24-dehydropollinastanol, in addition to several usual phytosterols, were also isolated and identified**.**[11] The wrightial, a new terpene and other phytoconstituents such as cycloartenone, cycloeucalenol were isolated identified by fractionation of methanol extract of the immature seed pods**.**[12] **The hexane extract of seed pods of *Wrightia tinctoria* was saponified and non saponifiable matter was fractionated with methanol gave a colorless substance, oleanolic acid.**[13] The five flavonoid compounds, Indigotin, Indirubin, tryptanthrin, isatin and rutin were isolated and identified from the leaves**.**[14] The bark is used as stomachic and in the treatment of abdominal pain and skin diseases**,**[15] as antidysenteric, antidiarrhoeal and antihaemorrhagic**.**[16] The bark is used in flatulence and bilious affections. A decoction of the leaves and bark is taken as a stomachic and in the treatment of abdominal pain**.**[17] The dried and ground bark is rubbed over the body in dropsy.

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Although *Abelmoschus manihot* and *Wrightia tinctoria* are reported to be used in a large number of Chinese traditional medicine preparations, there is no published report on the study of acute oral toxicity of both *Abelmoschus manihot* and *Wrightia tinctoria*. The acute oral toxicity test is the simplest, and often the first toxicity test to be conducted on a sample. A single, high dose of the test sample is given to each experimental animal and the mortality is observed; death within the observation period (usually of 14 days duration), whether caused by natural death or humane killing, is studied.[18] The findings of this study corroborated the need for a safety study on both the species used for primary health care in India. Such studies need to be carried out before the continued widespread use of some species provokes long-term and irreversible damage.

MATERIALS AND METHODS

Plant sample collection and identification

The fresh woody stem of *Abelmoschus manihot* and *Wrightia tinctoria* were collected from Toranmal hills of Maharashtra, India in September 2009 and February 2010, respectively. They were identified by Professor Dr. D. A. Patil of the S. S. V. P. S. Institute of Sciences, University North Maharashtra, India, and voucher specimens were deposited in the herbarium of the R. C. Patel Institute of Pharmaceutical Education and Research, University of North Maharashtra, India, with voucher numbers of PSJ/1235/09 (*Abelmoschus manihot*) and PSJ/1236/09(***Wrightia tinctoria***).

Chemicals

Anhydrous sodium sulfate was purchased from the Sigma-Aldrich Company, while Tween 80 and methanol were obtained from the Merck Company.

Preparation of extracts

The crude extracts were prepared as previously described.[19] Briefly, the fresh woody stems of *Abelmoschus manihot* and *Wrightia tinctoria* were washed, dried, and ground to a fine powder, using a blender. The dried, ground stems were then soaked in methanol (1.5 L) for three days, at room temperature. The solvent-containing extract was then decantered, dried with anhydrous sodium sulfate, and filtered. The extraction of the ground leaves was further repeated (2x) with methanol (1.5 L each time). The filtrates from each extraction were combined and the excess solvent was evaporated (Buchi, Rotavapor, Switzerland) under reduced pressure, using a rotary evaporator, to give a dark green crude methanol extract.

Test species

The experiment was performed on healthy ICR mice (five weeks of age, body weight 23-28 g), obtained from the National Toxicological Centre, University of Pune, India. The female mice were confirmed nulliparous and non-pregnant. The mice were assigned to five dosage groups and one control group with 10 mice (five male and five female) for each test group. The weight variation in the mice used did not exceed ± 20% SD of the mean body weight of each sex. The experimental procedures involving the animals were approved by the University of Malaya Animal Experimental Ethnics Committee [Ethical number: RCPIPER/IAEC/2008-09/53(R)] before commencing the study.

Procedure of acute oral toxicity

The acute oral toxicity of the crude methanol extracts of both *Abelmoschus manihot* and *Wrightia tinctoria* species were evaluated in mice using the procedure described by the OECD (Organization for Economic Co-operation and Development), with some modifications. The mice were housed in suspended, stainless steel, wire-mesh cages in an experimental animal room. The temperature was maintained at 23 ± 3 °C and the relative humidity was 50-60% before and after treatment with the extract. The animal room was artificially illuminated (fluorescent light) with an approximate 12-hour light/ dark cycle. The mice were acclimatized to the laboratory conditions for at least five days prior to commencement of the experiments. The mice were randomly selected for use in the study and marked to provide individual identification. Conventional mouse diets, with unlimited supply of drinking water, were available *ad libitum*, except during the fasting period. The mice were fasted approximately 12 hours prior to dosing, but they had free access to drinking water. Before and after treatment with the extract, the mice were caged in groups by sex and dose levels. The extracts were suspended in a vehicle (10% Tween-80 in distilled water). A stock concentration of 200 mg/ml was prepared and the mice were administrated with 0.2 ml of the extract for every 10 g of mice body weight. The mice were administered with doses of 500, 1000, 1500, 2000, and 2500 mg/kg of extracts. Food was started for the animals approximately three to four hours after dosing. The mice were observed carefully for any signs of toxicity in the first four hours after the treatment period, and daily thereafter for a period of 14 days.[20] Observations for mortality, signs of illness, injury, pain, distress, allergic reactions, changes of outer appearance, behavioral alterations (i.e., ataxia, hyperactivity, hypoactivity), and general stimulation or sedation were conducted twice daily. The observations were recorded systematically; individual records were maintained for each mouse.[21]

RESULTS AND DISCUSSION

Extraction yield of *Abelmoschus manihot* and *Wrightia tinctoria*

Solvent extraction is the most popular method used in sample preparation. The yields from methanol extracts of

Abelmoschus manihot and ***Wrightia tinctoria*** are shown in [Table 1]. Before extraction, the plant material needs to be dried to avoid the presence of water in the extracts. The percentage of crude methanol extract yield is based on the weight of dried and ground plant materials. Methanol is used as the extraction solvent due to its polarity and its known ability to extract compounds such as, phenolics, flavonoids, and other polar materials.

Acute oral toxicity assessment of *Abelmoschus manihot* and *Wrightia tinctoria* crude extracts

Investigation of acute toxicity is the first step in the toxicological analysis of herbal drugs.[22] Overall, animal models have a good predictability for human toxicities of around 70-80%.[23] Generally, it is possible to get the first hints on complex toxicities by applying in vivo methods, as information on some toxic manifestations cannot be assessed by in vitro cytotoxicity methods.[24] Toxic manifestations that affect the entire organism such as pain, distress, allergic reactions, changes in outer appearance, behavioral alterations, and general stimulation or sedation can be detected by in vivo assays. However, the detection of effects on vital functions (cardiovascular, central nervous, and respiratory systems) is usually not assessed in acute toxicity studies.

Acute oral toxicity was undertaken in the present study to determine the safety parameters of the leaves of *Abelmoschus manihot and Wrightia tinctoria*. Mortality, clinical signs, gross findings, and body weights of mice were observed and measured for 14 days after the oral administration of crude methanol extracts to both species. The crude methanol extracts were used in this acute oral toxicity study to ensure that all components in the extract were included.

The Table 2 shows the results of the acute toxicity of the crude extracts of *Abelmoschus manihot and Wrightia tinctoria*. For all doses tested for crude methanol extracts of *Abelmoschus manihot and Wrightia tinctoria*, there were no deaths reported. Throughout the 14-day observation period, there were no significant changes in behavior (i.e., ataxia, hyperactivity, hypoactivity) in any of the mice, nor did they produce any variations in the general appearance. They gained weight with no adverse clinical signs of toxicity at any dose.

Traditionally, the aim of the acute oral toxicity study was the estimation of LD_{50}. The LD_{50} value - defined as the statistically derived dose, which when administered in an acute toxicity test, is expected to cause death in 50% of the treated animals in a given period is currently the basis for toxicological classification of chemicals. For a classical LD_{50} study, laboratory mice and rats are the species typically selected. Often both sexes must be used for regulatory purposes.

As no deaths were found for all doses tested for crude methanol extracts of *Abelmoschus manihot and Wrightia tinctoria*, the LD_{50} values of crude *Abelmoschus manihot and Wrightia tinctoria* extracts were >2500 mg/kg. This indicated that both species did not cause any acute toxicity. According to the chemical labeling and classification of acute systemic toxicity, based on oral LD_{50} values, which were recommended by OECD,[25,26] the crude extracts of both species were assigned to class 5 (LD_{50} > 2000 mg/kg), which was termed as the lowest toxicity class (no label; unclassified). Oliver[27] pointed out that (i) the LD_{50} value was not an absolute value, but was an inherently variable biological parameter that could not be described in terms of accuracy, but only of precision, (ii) the LD_{50} value referred only to mortality and was illustrative of no other clinical expression of toxicity.

Table 1: Yield of methanol extracts of *Abelmoschus manihot* and *Wrightia tinctoria*

Plants	Sample/extracts	Weight (g) (%)
Abelmoschus manihot	Fresh sample	4525.10
	Dried and ground plant Material	752.52 (16.63)
	Methanol extract	86.53 g (11.50)
Wrightia tinctoria	Fresh sample	4525.10
	Dried and ground plant Material	648.44 (14.33)
	Methanol extract	66.14 g (10.20)

CONCLUSION

In view of the increasing popular consumption of medicinal plants as alternative therapy, it is necessary to conduct research to support the therapeutic claims and also to ensure

Table 2: Results of the potential toxic effect of the crude extracts of *Abelmoschus manihot* and *Wrightia tinctoria* in mice

Plants	Dose (mg/kg)											
	0[a]		500		1000		1500		2000		2500	
	M	F	M	F	M	F	M	F	M	F	M	F
A. manihot	0/5[b]	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
W. tinctoria	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

M = Male ICR Mice; F = Female ICR Mice; [a]control group (treatment without extract); [b]Number of animals dead/number of animals used

that the plants are indeed safe for human consumption. The present research findings have clearly met the objectives of the study. The result was in agreement with that of in vitro experiments, whereby, the crude extracts of *Abelmoschus manihot and Wrightia tinctoria* did not show cytotoxicity against normal MRC-5 cells.[19,28] Based on the outcome of acute toxicity in experimental mice, the crude extracts of both species could be regarded as safe in experimental mice. Further toxicity study over a longer period of time involving detection of effects on vital organ functions would ensure that the plants are safe for human consumption.

REFERENCES

1. Wills RB, Bone K, Morgan M. Herbal products: Active constituents, modes of action and quality control. Nutr Res Rev 2000; 13:47-7.
2. Kiritikar KR, Basu BD. Indian Medcinal Plants. 2nd ed. India: Allahabad; 1994. p.1606-09, 1783- 92.
3. Lai XY, Zhao YY, Liang H. Studies on chemical constituents in flower of *Abelmoschus manihot.* China J Chin Mat Med 2006; 31(19):1597-600.
4. Liang H, Lai X, Zhao Y, Bai Y, Wang B, Guo D. SPE-HPLC method for the determination of four flavonols in rat plasma and urine after oral administration of *Abelmoschus manihot* extract. J Chromatogr B Analyt Tech Biomed Life Sci 2007; 852(1-2):108-14.
5. Lai X, Liang H, Zhao Y, Wang B. Simultaneous determination of seven active flavonols in the flowers of Abelmoschus manihot by HPLC. J Chromatogr Sci 2009; 47(3):206-10.
6. Linlin WU, Xin-bo Y, Zhengming H, Hezhi L, Guangxia WU. In vivo and in vitro antiviral activity of hyperoside extracted from *Abelmoschus manihot* (L) medic. Acta Pharmacol Sin 2007; 28(3):404-409.
7. Wen JY, Chen ZW. Protective effect of pharmacological preconditioning of total flavones of *Abelmoschus manihot* on cerebral ischemic reperfusion injury in rats. Am J Chin Med 2007; 35(4):653-61.
8. Puel C, Mathey J, Davicco MJ, Lebecque P, Chanteranne B, Horcajada MN, Coxam V. Preventive effect of *Abelmoschus manihot* (L) medik on bone loss in the overiectomised rats. J. Ethnopharmacol 2005; 99:55-60.
9. **Jain PS, Bari SB. Analgesic activity of *Abelmoschus manihot* extracts. Int J. Pharmacol 2011; 1-5.**
10. Anonymous: The wealth of India: Raw Materials. India, New Delhi: Publication and Information Directorate CSIR; 1976.
11. Akihisa T, Ishtiaque A, Singh S, Tamura T, Matsumoto M. 14α-Methylzymosterol and other sterols from *Wrightia tinctoria* seeds. Phytochem 1988; 27(10): 3231-3234.
12. Ramchandra P, Basheermiya M, Krupadanam GLD, Srimannarayana G. Wrightial, a new Terpene from *Wrightia tinctoria*. J Nat Prod 1993; 56(10):1811-1812.
13. **Rao MN, Rao V, Nageswara M. Occurrence of oleanolic acid in the pods of *Wrightia tinctoria* br. Current Sciences 1968; 22:645-46.**
14. Muruganadam AV, Bhattacharya SK, Ghosal S. Indole and flavonoid constituents of *Wrightia tinctoria and W. tomentosa and W. coccinea*. Ind J Chem 2000; 39 B(2):125-131.
15. Shah GL, Gopal GV. Ethno medical notes from the tribal inhabitants of the north Gujarat (India). J Eco Tox Bot 1988; 6:193-221.
16. Singh B, Sharma MK, Meghwal PR, Sahu PM, Singh S. Anti-inflammatory activity of shikonin derivatives from *Arnebia hispidissima*. Phytomed. 2003; 10:375-80.
17. Stallard N. Optimal adaptive designs for acute oral toxicity assessment. J Statist Plann Inference 2006; 136:1781-99.
18. Sri Nurestri AM, Norhanom AW, Hashim Y, Sim KS, Hong SL, Lee GS, *et al*. Cytotoxic activity of *Pereskia bleo* (Cactaceae) against selected human cell lines. Int J Cancer Res 2008; 4:20-7.
19. Deciga-Campos M, Rivero-Cruz I, Arriaga-Alba M, Castaneda-Corral G, Angeles-Lopez GE, Navarrete A, *et al*. Acute toxicity and mutagenic activity of Mexican plants used in traditional medicine. J Ethnopharmacol 2007; 110:334-42.
20. **Shuid A N, Siang Lk, Chin TG, Muhammad, N, Soelaiman IN. Acute and Subacute Toxicity Studies of *Eurycoma longifolia* in Male Rats. Int J Pharmacol 2011; 7:641-646.**
21. **Paul NA, Memfin E, Tony Waka U, Jude O, Augustine Bassey L. Acute toxicity potential of methanolic extract of *Smilax kraussiana* leaves in rats. Int J Pharmacol 2006; 2:463-466.**
22. Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, *et al*. Concordance of the toxicity of pharmaceuticals in humans and in animals. Reg Toxicol Pharmacol 2000; 32:56-67.
23. Kola I, Landis J. Can the pharmaceutical industry reduce attrition rates? Nat Rev Drug Discov 2004; 3:711-6.
24. Ukelis U, Kramer PJ, Olejniczak K, Mueller SO. Replacement of *in vivo* acute oral toxicity studies by *in vitro* cytotoxicity methods: Opportunities, limits and regulatory status. Reg Toxicol Pharmacol 2008; 51:108-18.
25. OECD (Organization for Economic Co-operation and Development). Harmonized Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances. Paris: OECD; 1998.
26. Walum E. Acute oral toxicity. Environ Health Perspect 1998; 106:497-503.
27. Oliver JA. Opportunities for using fewer animals in acute toxicity studies. In: Chemicals testing and animal welfare. Sweden: The National Chemicals Inspectorate; 1986. p.119-42.
28. **Lingaraju GM, Hoskeri JY, Krishna V, Suresh babu P. Analgesic activity and acute toxicity study of *Semecarpus anacardium* stem bark extracts using mice. Pharm Res. 2011; 3(1):57-61.**