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Acute Oral Toxicity Assessment of Aqueous Extract of Streblus asper Root in Sprague Dawley Rats according to OECD 420 TG

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Abstract The growing interest in the use of medicinal plants for treatment and healthcare necessitates thorough investigations into their safety profiles, many of which remain scarce. This research evaluates the acute toxicity of *Streblus asper* (SA) root aqueous extract, adhering to the OECD 420 testing guideline. Initially, a preliminary study administered increasing single doses of the extract, ranging from 5 up to 2000 mg/kg body weight, via oral gavage to female Sprague-Dawley rats. Following the determination of an appropriate starting dose, the main study included four additional rats. Over 14 days, subjects were monitored for mortality, toxicity signs, body weight and food consumption, followed by gross pathology analysis at the study's conclusion. The findings revealed no mortality or toxicity signs, consistent weight gain corresponding to food intake and no adverse effects on vital organs. Consequently, the study infers that the LD_{50} of SA root extract exceeds 2000 mg/kg body weight in rats, indicating a potentially safe profile for therapeutic applications. This research contributes valuable data on the safety of medicinal plants, supporting their integration into healthcare practices.

Key Words Acute oral toxicity, Streblus asper, LD50, OECD 420 TG

INTRODUCTION

Medicinal plants have been a cornerstone in the treatment of diseases and the improvement of general health for thousands of years. Their role in healthcare is both ancient and invaluable, bridging the gap between traditional remedies and modern medicine. This introductory discussion seeks to explore the enduring use and significance of medicinal plants, highlighting their continuous contribution to health and wellness across cultures. Approximately 80% of the global population relies on medicinal plants for their daily health care [1]. Evidence has shown that medicinal plants hold significant promise in treating various diseases, yet certain plants can become harmful when overdosed or may interact adversely with conventional medications [2]. Therefore, ensuring the safety of medicinal plants must be a top priority.

Streblus asper Lour, commonly recognized as a shrub tree, is predominantly utilized in landscaping across various regions. These plants are native to tropical countries and mainly distributed in Malaysia, Thailand and India. The plants are traditionally used to treat various ailments including dysentery, leprosy, piles and elephantiasis. Recent years have witnessed an upsurge in research focused on the pharmacological properties of *Streblus asper* extract, employing a range of in vitro and in vivo testing models. The different parts of the plants were found to have antimicrobial, antifilarial, antiallergic and anticancer [3]. For instance, the root extract showed chemopreventive effect on osteosarcoma and tongue carcinoma cell line by inducing apoptosis [4].

Phytochemical profile of the *Streblus asper* indicates the presence of different types of bioactive compounds including lignans, triterpenoids and flavonoids [5]. Cardiac glycosides are among the isolated compounds from *Streblus asper* extract [6]. Cardiac glycosides has side effects which cause poisoning to the gastrointestine and heart [7]. Therefore, the safety assessment of these plants is necessary to be conducted through a pre-clinical study.

Given the scarcity of experimental insights into the toxicity of *Streblus asper* root, our study embarked on a pioneering investigation to elucidate the potential toxicological impact of its aqueous extract on female

Sprague-Dawley rats. This study aimed to assess the potential toxic effects of *Streblus asper* root aqueous extract in female Sprague-Dawley rats after a single oral administration, in line with the OECD 420 test guideline for a 14-day acute toxicity study.

METHODS

Animals

Sprague-Dawley rats were used in this study. Female rats (nulliparous, non-pregnant) at age around 6-8 weeks were purchased from M_CLEA Bioresources Co, Ltd (Samutprakarn, Thailand). Each animal was housed in single housing in individually ventilated cages (IVC) system (Tecniplast Sealsafe Plus, Italy) with corncob bedding, feed and an unlimited supply of drinking water available *ad libitum*. The environmental conditions in the animal room were maintained at a temperature of 22±3°C, with relative humidity between 30-70% and a 12-hour light/dark cycle.

Animals were randomly selected and kept in their cages for acclimatization to the room conditions for one week prior to dosing. This study was approved by IPharm Animal Ethics Committee (IPAEC) with the ethical clearance code NIBM/IPharm/PTR(S)100-7/74(2017-006) for the acute oral toxicity study.

Test Substance

In this study, an aqueous extract of *Streblus aspe r* root was used as the test substance. The *Streblus asper* plants were obtained from a supplier in Tasek Gelugor, Penang, Malaysia. The plant's extract was prepared using the boiling method introduced by Seeni *et al.* [4]. Briefly, *Streblus asper* roots were harvested from the whole plants and washed using tap water. The cleaned roots were air-dried for several days before cut into smaller pieces and further ground into powder using IKA A11 basic grinder (Staufen, Germany). The ground roots were boiled in distilled water for 10 minutes. The plants' water extract was sieved using Whatman Grade 1 filter paper and then centrifuged at 10,000 rpm for 30 minutes to remove agglomerates and impurities. The recovered supernatant of the extract was frozen at -80°C and further lyophilized using Christ Alpha 1-4 LSC basic freeze dryer (Osterode, Germany). The lyophilized extract was a fine powder with a dark brown colour.

Preparation and Administration of Doses

The test substance was freshly prepared during the administration day. It was dissolved in reverse osmosis water to achieve concentration at 5, 50, 300 and 2000 mg/kg body weight. The administration was through oral gavage, with a dosing volume of 1 ml per 100 g of the animal's body weight. The animals were fasted overnight before dosing but water was provided throughout the fasting period. The animals were initially weighed prior the administration procedure following the fasting period. Food was then withheld for an additional 3-4 hours after administration.

Acute Oral Toxicity Study

An acute oral toxicity test with single dose of *Streblus asper* root aqueous extract was conducted via oral administration in female Sprague-Dawley rats, following the OECD Guideline for Testing of Chemicals: Acute Oral Toxicity - Fixed Dose Procedure (Document 420, OECD, 2001). The sighting phase served as the initial part of the study, aimed at selecting the appropriate starting dose for the main study. In this phase, a single dose of 5 mg/kg body weight of the test substance was administered to one animal. Clinical observation on the animal was then performed at 0.5, 1, 2, 3 and 4 hours after dosing. After 24 hours of observation and the animal survived, a new animal was administered with the next higher dose (50 mg/kg body weight) and observed in the same manner. These steps were repeated for the dose 300 and 2000 mg/kg body weight in a sequential manner. Once the animal dosed at the highest dose survived in the sighting study, the main study was carried out with four additional animals. The animals were administered with a single dosing of the appropriate dose level determined from the sighting study. All animals in both studies were monitored daily for a period of 14 days.

Clinical Observations

Clinical observations on individual animals were performed at 0.5, 1, 2, 3 and 4 hours after dosing and once daily thereafter, for a total of 14 days. Morbidity and mortality were checked twice daily. Observations included changes in skin and fur, eyes, mucous membranes, respiratory function, autonomic and central nervous systems, somatomotor activity and behavior patterns. Particular attention was given to signs such as tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. Any signs of toxicity or deaths were recorded daily throughout the study period.

Body Weight

The body weight of each individual animal was measured just before the administration of the test substance (day 0) and weekly thereafter (on days 7 and 14). The mean weekly body weight was then calculated.

Food Consumption

Food consumption was measured starting from the first day of dosing and daily thereafter for a total of 14 days. Before dosing, animals overnight fasted for no more than 14 hours. Mean daily food intake was then calculated.

Gross Pathology

On day 14, the animals were euthanized using carbon dioxide overdose. All animals underwent an examination of gross necropsy examination, which included the external surfaces of the body, all orifices, cranial cavity, external surfaces of the brain, nasal cavity, thoracic, abdominal, pelvic cavities and viscera. All pathological changes were documented for each animal and only those organs exhibiting signs of gross pathology were selected for microscopic examination.

Statistical Analysis

Mean weekly body weight and daily food intake were measured by descriptive analysis using SPSS 16.0 statistical software.

RESULTS

Sighting Study

The animal dosed at 5 mg/kg body weight survived for 24 hours after administration. Consequently, a further test was conducted with a new animal at a dose of 50 mg/kg body weight and this animal also survived the dosing period. Both animals administered with the subsequent dose levels of 300 and 2000 mg/kg body weight survived as well. All animals remained healthy and active, exhibiting normal clinical signs until the end of the study (day 14). No unusual behavioral changes or severe clinical toxicities were observed in any of the animals treated with the test substance. Additionally, no gross pathological findings were noted in any animal during the necropsy. Overall, there were no recorded mortalities or abnormal clinical signs among all the dosed animals, as detailed in Table 1.

Main Study

Since the highest dose (2000 mg/kg body weight) of the test substance did not result in any fatalities, the main study involved four additional animals that received a single dose at the same level and were monitored for mortality and clinical signs until day 14. All animals survived, remaining healthy and active with normal clinical signs. No unusual behavioral changes or severe clinical toxicities were noted during the observation period. Additionally, no gross pathological findings were observed in any of the four animals during necropsy (Table 1). Therefore, the acute oral LD50 of the *Streblus asper* root aqueous extract in female Sprague-Dawley rats was estimated to be greater than 2000 mg/kg body weight.

Body Weight and Food Consumption

The body weight and food consumption of the animals treated with *Streblus asper* root aqueous extract in both sighting and main study were found to be normal. All animals gained weight during the studies as seen in Figure 1 with mean body weight gain ranging from 16 to 27 g per week. The weight gain of the animals was consistent with their daily food consumption. All the animals consumed food with mean daily intake ranging from 18 to 25 g per day (Figure 2).

Table 1: Mortality, clinical signs and gross pathology observations of animals treated with Streblus asper root aqueous extract in sighting and main study.

Study type	Dose (mg/kg BW)	No. of Animals	Sex	Mortality	Clinical Sign	Gross Pathology
Sighting Study- Step I	5	1	Female	0	Normal	No Lesions
Sighting Study- Step II	50	1	Female	0	Normal	No Lesions
Sighting Study- Step III	300	1	Female	0	Normal	No Lesions
Sighting Study- Step IV	2000	1	Female	0	Normal	No Lesions
Main Study	2000	4	Female	0	Normal	No Lesions

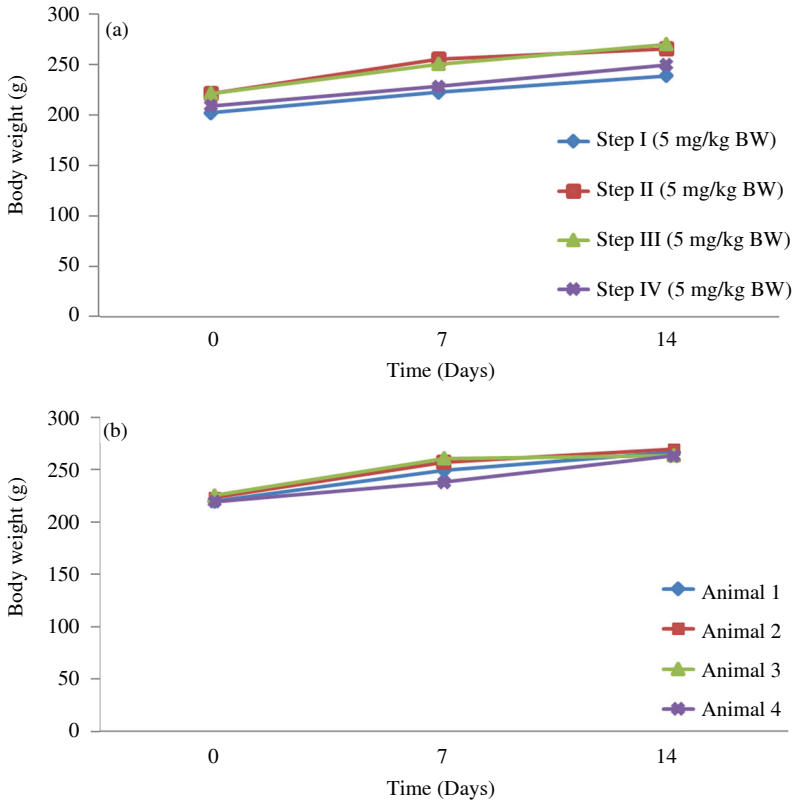

Figure 1(a-b): Body weight of animal treated with *Streblus asper* root aqueous extract in (a) Sighting study and (b) Main study for 14 days

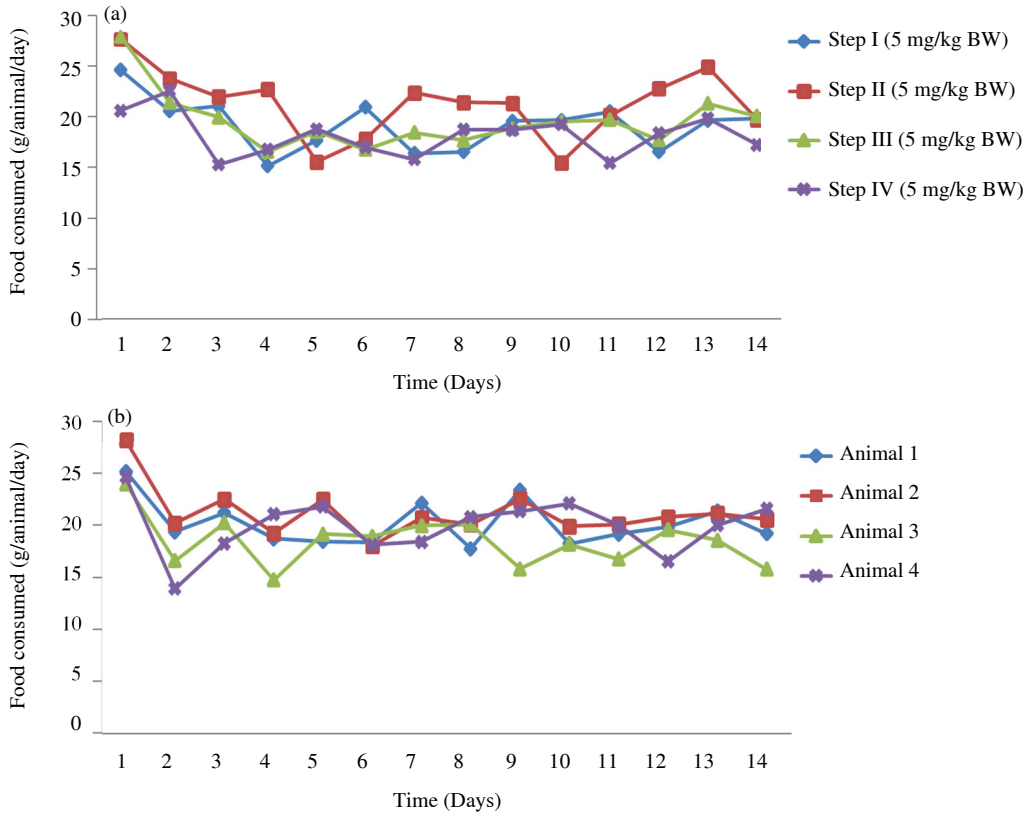

Figure 2: Food consumption of animals treated with *Streblus asper* root aqueous extract in (a) Sighting and (b) Main study for 14 days

DISCUSSION

Though *Streblus asper* is widely utilized in traditional medicine for treating various ailments, there is limited scientific data regarding the safety of its root extract. The main objective in assessing the safety of the plant is to identify its nature and any adverse effect it may cause to humans, as well as to determine the level of exposure at which these effects are observed. In the present study, we reported an evaluation of the safety and toxicological profile of the aqueous extract of *Streblus asper* root through conduction of acute oral toxicity studies in female Sprague-Dawley rats. In accordance with the OECD 420 test guideline, this study exclusively utilizes female animals because they are considered more vulnerable and possess a lower capacity for detoxification compared to males [8]. Although there is some variation in term of gender sensitivity, females are more sensitive in most cases [9].

In the sighting study, preliminary screening of dose levels up to 2000 mg/kg of *Streblus asper* root aqueous extract caused no mortality and no clinical signs of abnormalities in the animals. The weight gain of the animals was consistent with their daily food consumption. Even with further assessment in the main study using the highest dose level of the extract, the animal survived and no clinical signs of abnormalities were observed in the four additional animals. Thus, the acute oral LD_{50} for *Streblus asper* root aqueous extract was assumed to be greater than 2000 mg/kg body weight. According to OECD criteria under Globally Classification System (GHS) for chemical substances and mixtures, any substances with LD_{50} >2000-5000 mg/kg are categorized under category 5 [8] and considered safe. Safety studies on the other parts of *Streblus asper* have also been reported. For instance, leaf extract of *Streblus asper* was found to be non-toxic at a dose of 2000 mg/kg in mice [10] and in rats [11,12], which are consistent with finding of this study. Seeni *et al.* [4] reported that stem bark extract of *Streblus asper* showed no signs of toxicity or mortality in rats at the highest tested dose of 1500 mg/kg. These data demonstrate the overall safety of all parts of *Streblus asper* for the consumption.

Measurement of feed consumption and body weight of the animals served as indicators of health condition of the animals in the toxicity study [13]. Body weight gain over time is influenced by consistent feed intake, as highlighted by Kuriyan *et al.* [14], with appetite controlling the desire for intake. In this study, after a single administration of *Streblus asper* root aqueous extract, the animals maintained their feed consumption, as indicated by the body weight gain over a 14-day observation period. This suggests that the extract does not interfere with the animals' appetite or metabolism. These findings are consistent with a previous report indicating no significant changes in animals body weight following repeated administration of *Streblus asper* leaf extract, even over an extended period [11].

Streblus asper is rich with cardiac glycosides. More than 20 cardiac glycosides have been isolated from the root of

Streblus asper [15-18]. Cardiac glycosides are known for their toxicity on the cardiovascular, neurologic and gastrointestinal systems [19]. However, our finding suggests that cardiac glycoside presence in the extract may not contribute to the toxicity in the tested animals. This possibly is due to its low amount in the collected plants, a variation influenced by geographical distribution. Besides, antagonistic interactions between bioactive compounds within the plant extract may hamper the toxicity of cardiac glycosides from the sum of their effects when applied separately [20]. Further evidence indicates that the presence of certain cardiac glycosides in *Streblus asper* is non-toxic [21].

CONCLUSIONS

The findings of the present study demonstrate that a single oral dose of 2000 mg/kg of *Streblus asper* root aqueous extract did not produce acute oral toxicity in Sprague-Dawley rats. Its estimated minimal lethal dose LD_{50} for rats is greater than 2000 mg/kg. Longer-term study such sub-acute 28-days repeated dose is proposed for further investigation on the estimation of a no-effect dose level. Overall, this acute toxicity study contributes important insights into the toxicity profile of *Streblus asper*, providing a valuable foundation for the design of future pre-clinical research.

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Ethical Statement

This study was approved by IPharm Animal Ethics Committee (IPAEC) with the ethical clearance code NIBM/IPharm/PTR(S)100-7/74(2017-006) for the acute oral toxicity study.

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