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Toxic effects of *sapium indicum* (willd.) fruits on animal model

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Abstract

The present study deals with toxic effects of petroleum ether (40°-60°C) and water-soluble alcoholic extracts of the fruits of *Sapium indicum* (Willd.) on laboratory animal model. The petroleum ether (40°-60°C) extract administered through intravenous route in mice, and through oral routes in rats exhibited toxic effects. The 24-hr LD_{50} value of the extract was found as 816.58 $\mu g.kg^{-1}$ and 208.93 $mg.kg^{-1}$ body weight in mice and rats, respectively. On the other hand, the experimental rats treated orally with the water-soluble alcoholic extract exhibited no mortality. The results revealed that the petroleum ether (40°-60°C) extract of the fruit is more toxic than organochlorine compounds having a prospect of using it as an alternative source of bio-pesticides.

Keywords: *Sapium indicum* (Willd.), toxic response, mice, rat, 24-hr LD_{50}, bio-pesticide.

Introduction

The plant, *Sapium indicum* (Willd.) (syns *Excoecaria indica* Müll. - Arg.), an evergreen mangrove associated species of Euphorbiaceae family, ranges from South Asia (Bangladesh, South and East India), throughout Southeast Asia (Malaysia, Thailand, Indonesia, Sri Lanka) to Solomon Islands [1-3]. It is known as Melgota, Harua, Batul, Bolas or Urmel in Bangladesh [1], Ligura or Gurah in Malaysia [2], Mock Willow (English), Ai Tui, Ai Tohi or Ai Pue (Indonesia), Samo Thale (Thailand) [4]. Fruit is round, woody capsule, 2.5-3.0 cm in diameter, almost black and 3-seeded [4]. Seed is ellipsoid, slightly compressed, smooth and plae-coloured [3]. Children like to play marbles with the fruits [2, 4]. Latex of the young fruit wall contains aesculetin, which is caustic and blisters the skin [2]. Ripe fruits are purgative and poisonous in nature, and are used intoxicating fish [3].

There are numerous reports published on different aspects of the plant including chemical analysis of the fruits [5-13]. The fruit is reported to exhibit antimicrobial [12, 14], insecticidal [15] and piscicidal [16, 17] effects. There has been reported no toxicological study using different crude extracts of the fruit on animal model. Therefore, the present study was determined to investigate toxicological properties (i.e, LD_{50} values and behavioural responses) of different crude extracts of the fruits of *S. indicum* (Willd.) on laboratory animals.

Materials and Methods

Collection of sample

Fresh fruits (10 kg) of *S. indicum* (Willd.) were collected from Nayarhat Village of Chittagong, Bangladesh. The sample was authentically identified by Mr. Jashim Uddin Chowdhury, Senior Scientific Officer, Botany Division, BCSIR Laboratories, Chittagong, Bangladesh. The voucher specimen was deposited to the herbarium of BCSIR Laboratories.

Preparation of extracts

The fruits were allowed to dry under sun for 4 days. The dried fruits were then pulverized into fine granules with the help of a power-driven grinder. The granular matter was soaked in 10.0 L of petroleum ether (40°-60°C) (Sigma-Aldrich, USA) (1:5, w/v) for 3 days in a glass container. The filtrate was collected and evaporated through a rotary vacuum evaporator (Eyela-1000S, Thermo Fisher Scientific, USA) at 50 ± 5° C. The crude extract thus obtained was collected in a pre-cleaned screw-capped bottle (250 mL) and stored in a refrigerator, which was used subsequently as the test substance under the code name of PSE.

The residual part was dried and soaked in 9.0 L of Methyl Alcohol (Sigma-Aldrich, USA) for three days in a glass container. Crude extract was obtained by following the same procedure as illustrated earlier. The crude extract was then subjected to serial fractionation

as illustrated by [18]. The final fractions (water-soluble extract and alcohol-soluble extract) were collected into two separate pre-cleaned screw-capped bottles (250 mL and 50 mL respectively), dried in an oven for six hours at temperature 30±5°C and stored in a refrigerator. Only the water-soluble extract was used as the test substance under the code name of WAE. Only a few amount of alcohol soluble extract was produced and hence it was not tried in the present study.

Collection of laboratory animals

Mice (*Mus domesticus*) and rats (*Rattus norvegicus*) were collected from the Animal Breeding Centre, BCSIR Laboratories, Chittagong. They were kept under normal laboratory condition for three days while normal food was supplied them. Before performing the toxicity test on animal model, ethical clearance was made from the BMRC (Bangladesh Medical Research Council), Dhaka.

Preparation of test substances for administration

The test substances were prepared in accordance to Turner [19] for administering to the laboratory animals. Pure coconut oil was used as vehicle in the case of PSE whereas distilled water in the case of WAE. Standard guidelines were followed for the toxicity testing [19].

Experiment on mice with the PSE

After preliminary exploratory test, 30 male mice (mean weight 23.5±1.5 gm) were selected and equally divided into six groups, out of which one group was taken as the control group and treated with the vehicle only. The rest five groups were considered for acute toxicity test. Mice were treated with the PSE at the dose level of 50 to 1500 $\mu g.kg^{-1}$ body weight through intra-venous injection. Mortality within 24 hours along with some behavioural observations was made following the method of Irwin [20].

Experiment on rats with the PSE

After preliminary exploratory test, acute toxicity test on 25 rats (mean weight 170 ± 5 gm) was performed following the same procedure as stated earlier. The five testing groups were treated orally with the PSE while the control group consisting 5 rats was treated with the vehicle only. All groups were treated orally with the PSE at the doses ranging from 175 to 275 $mg.kg^{-1}$ body weight with the help of a stomach tube. Mortality rates, behavioural observations were ascertained following the same method as illustrated earlier.

Experiment on rats with the WAE

The test was carried out following the same procedure as stated before wherein the five testing groups of rats (mean weight 170 ± 5 gm) were treated orally with the WAE at the doses of 100, 250, 500, 1000 and 2000 $mg.kg^{-1}$ body weight with the help of a stomach tube. The control group consisting 5 rats was treated with the vehicle only. Mortality rates and behavioural observations were made for 24 hours following the same method as described earlier.

Statistical analysis

Mortality data were subject to probit analysis for determining the 24-hr LD_{50} value [21]. The χ^2 test was performed to test the homogeneity of the experimental data.

Results and Discussion

Biological behavior, a sensitive indicator of neuronal function, is the integrated sum of activities mediated by the nervous system. Toxicants can alter behavior in varied ways [22]. Irwin protocol is widely used for systematic evaluation of toxic responses in laboratory animals. In line with this protocol in the present investigation, all the test substances exhibited toxic responses on the experimental animals. The PSE at 100 $\mu g.kg^{-1}$ body weight dose level produced toxic signs in mice (Table 1).

Table 1: Gross observation on the experimental mice treated intravenously with the PSE.

Gross observation	Normal score	Time (hour) 1	3	6	Overnight	24
Alertness	4	2	2	3	--	4
Stereotypy	0	3	3	4	--	3
Passivity	0	2	2	2	--	3
Restlessness	0	3	4	4	--	3
Irritability	0	4	5	5	--	4
Startle response	0	2	2	1	--	0
Urination	0	3	2	2	--	1
Salivation	0	5	6	6	--	4
Writhing	0	3	2	2	--	0
Respiratory rate	4	8	7	7	--	6
Lacrimation	0	6	6	5	--	4
Soft fecal pellets	0	2	2	1	--	0

(-- not observed)

From the probit mortality analysis, the 24-hr LD50 value of the PSE for mice was found 816.58 $\mu g.kg^{-1}$ body weight (Figure 1) with the fiducial limit value of 816.58±1.55 at 0.1% level of confidence. The χ^2 value was found significant at 0.1% level, which is indicative of homogeneous relationship between the PSE doses and mortality rates of the treated mice.

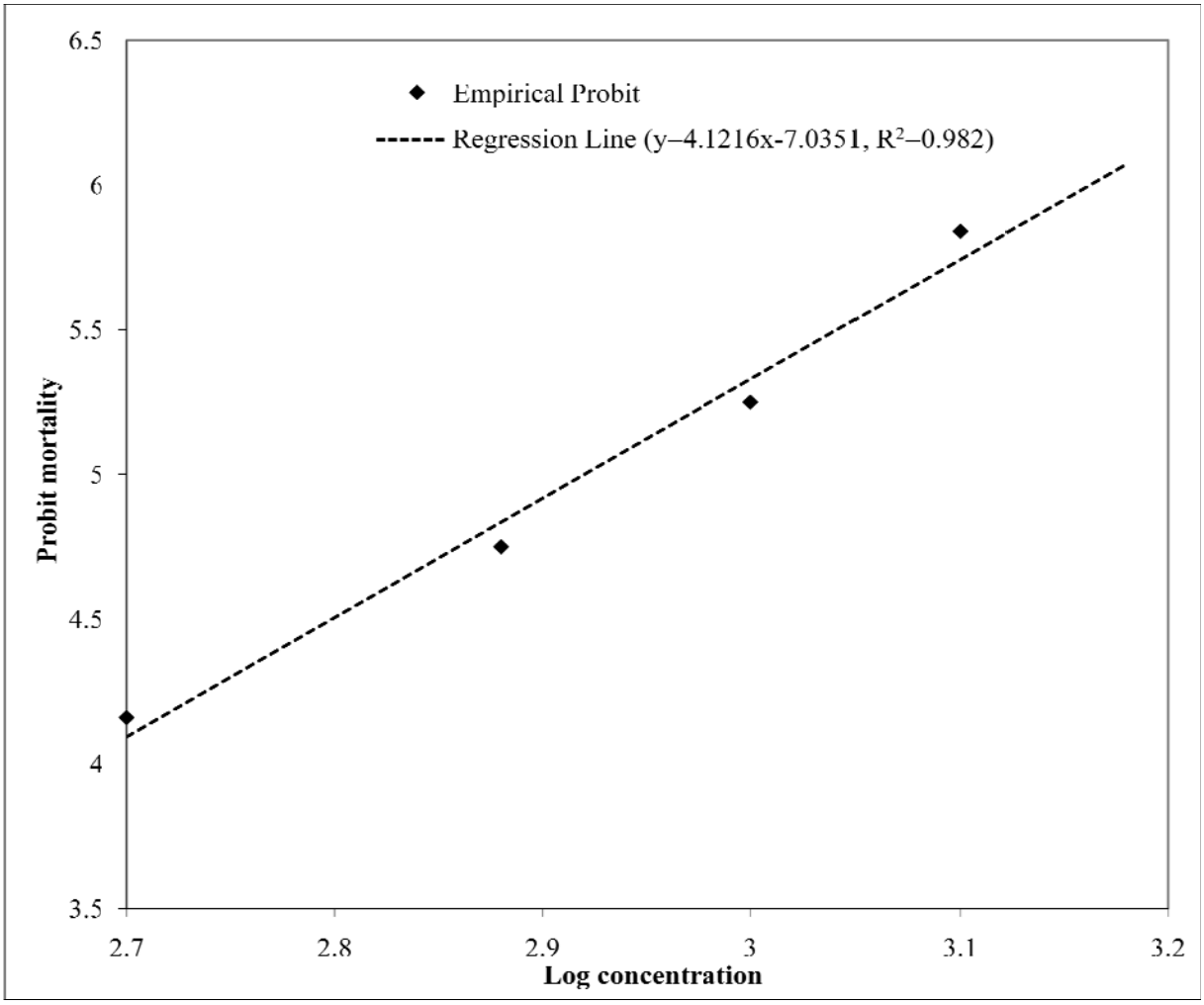

Figure 1: Probit mortality analysis of the PSE in mice.

Table 2: Gross observation on the experimental rats treated orally with the PSE.

Gross observation	Normal score	Time (hour) 1	3	6	Overnight	24
Alertness	4	2	3	3	--	4
Stereotypy	0	2	2	3	--	2
Passivity	0	2	2	2	--	3
Restlessness	0	3	4	4	--	3
Irritability	0	4	4	5	--	4
Startle response	0	2	1	1	--	0
Urination	0	3	3	2	--	1
Salivation	0	5	6	6	--	4
Writhing	0	2	2	1	--	0
Respiratory rate	4	7	7	6	--	6
Lacrimation	0	6	6	5	--	4
Soft fecal pellets	0	3	3	2	--	0

(-- not observed)

Almost similar toxic symptoms were observed in the experimental rats treated with PSE at 225 mg.kg^{-1} dose level (Table 2). The 24-hr LD_{50} value of the PSE for rats was found 208.93 mg.kg^{-1} body weight (Figure 2) with the fiducial limit value of 208.93±1.14 at 1% level of significance. The χ^2 value was found significant at 1% level indicating the fact that the PSE doses and mortality rates of the treated rats were homogeneous.

On the other hand, the rats treated orally with the WAE at 2000 mg.kg^{-1} body weight showed toxic symptoms to a lesser extent than in the other case (Table 3). But no animal was found dead at the test dose level even after 24 hours of observation.

An earlier study reported the toxicological profile of the juice from the fruits of *S. indicum* [23]. The authors also observed similar toxic responses in albino rats that were observed in the present study. Therefore, the present findings are corroborated with the previous study [23]. It was also reported that the juice of the fruits of *S. indicum* (Willd.) is highly toxic to the albino rats when administered through intra-peritoneal route, but no apparent toxicity does it

produce when administered through oral route. The 24-hr LD_{50} value was reported as 25-50 $mg.kg^{-1}$ body weight when the juice was administered to the albino rats intravenously [23]. However, the PSE in the present investigation was less toxic than the juice of the fruits.

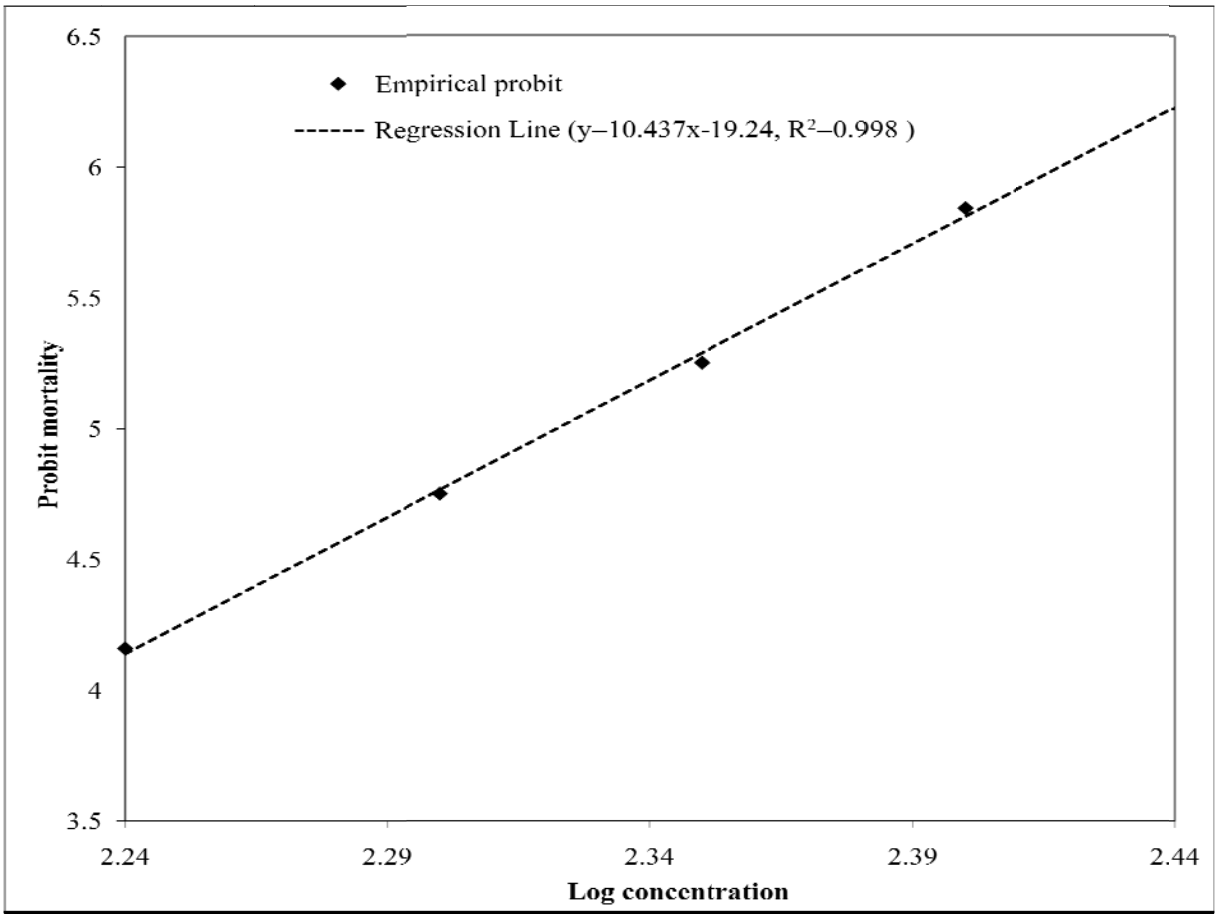

Figure 2: Probit mortality analysis of the PSE in rats.

Table 3: Gross observation on the experimental rats treated orally with the WAE.

Gross observation	Normal score	Time (hour)				
		1	3	6	Overnight	24
Alertness	4	3	3	4	--	4
Stereotypy	0	2	2	1	--	0
Passivity	0	1	1	0	--	0
Restlessness	0	2	2	1	--	0
Irritability	0	3	3	2	--	0
Startle response	0	1	1	0	--	0
Urination	0	2	2	1	--	0
Salivation	0	3	3	2	--	0
Writhing	0	1	1	0	--	0
Respiratory rate	4	5	5	5	--	4
Lacrimation	0	2	2	1	--	0
Soft fecal pellets	0	2	2	1	--	0

(-- not observed)

Fruits of *S. indicum* (Willd.) are known to posses many active chemical compounds. Diterpene ester alkaloid (4α-sapinine) [6], diterpenes (4-deoxyphorbol, 4∞-deoxyphorbol aldehydes) [9], phorbol ester (Sapintoxin A) [8], esters of deoxyphorbol (Sapintoxins B and C) [10], aliphatic esters of the tigliane nucleus (derivatives of 4-deoxyphorbol), Sapatoxins A, B and C [11] were isolated from the fruits. Some of these chemicals were reported to exhibit toxic responses. Esters of phorbol and related polyols are known to cause skin irritation [24]. The sapintoxin A was reported to be a rapidly acting proinflammatory agent on mammalian skin, and it significantly increased perfusion pressure in rat model [25]. Tigliane nucleus (4-deoxy-phorbol derivatives) possesses tumor promoting and irritant activities [26 27]. Phorbol esters of other plant origin (e.g., *Jatropha curcas*) were reported to exhibit toxic symptoms and effects in rodents [28].

Restlessness and irritability are known to be anticholinergic effects of poisonous chemicals [29]. Toxic chemicals (e.g., pyrethoids) exhibit increased startle response and profuse salivation in rats

[30], and those in addition to lacrimation, increased urination, and diarrhoea are features of parasympathetic stimulation . It is therefore, suggested from the gross observations (Table 1, 2 & 3) that the test substances might have the properties of CNS depressant, myorelaxation, muscarinic activity and respiratory analeptics at different levels of efficacy as well as caused visceral changes in the treated animals. Nevertheless, the PSE is more toxic to mice and rats than the WAE. It was reported that deaths from toxic exposure occur due to respiratory failure resulting from inhibition of respiratory centers in the brain stem [31, 32]. The cause of mice and rats death in the present study might be due to the presence of the active chemical principle, saponin [3, 16, 17] and/or sapintoxins [8, 10] present in the fruits.

Table 4: Toxicity of organochlorine pesticides on rats treated orally (Gaines, 1969).

Organochlorines	24-hr LD_{50} ($mg.kg^{-1}$)
p/,p/-DDT	113
DDE	880
DDA	740
Methoxychlor	5000-7000
Aldrin	39
Dieldrin	46
Endrin	18
Heptachlor	100
Chlordane	335
Lindane	88
Mirex	740

Organochlorine compounds were reported to be toxic to the experimental animals at various doses (Table 4). Although the experimental condition is not similar to the present study, a comparative study can be made with the findings of Gaines [33]. It becomes evident that the PSE is more toxic to mice and rats than some organochlorine compounds. Therefore, the fruits of *S. indicum* (Willd.) may be a useful source of bio-pesticide for reducing the use of long persisting, non-biodegradable organochlorne pesticides, which are harmful for all the components of our environment. Further study in this instance is necessary to evaluate its potential aspects at the field level.

Conclusions

In the present study, acute toxicity and behavioural responses in mice and rats were observed with the crude extracts of *Sapium indicum* (Willd.). The crude extract of petroleum ether (40°-60°C) was found to exhibit toxic effects in mice and rats with the 24-hr LD_{50} value of 816.58 $\mu g.kg^{-1}$ and 208.93 $mg.kg^{-1}$ body weight respectively. It is assumed that the fruit can be an effective source of biopesticide. However, further investigation is needed to explore in this respect.

Authors' Contribution

MSR conceived the study, carried out the design, data analysis and interpretation of data, drafting of manuscript.
TM participated in the design of the study, involved in data analysis and interpretation, drafting of manuscript, revising it critically for an important intellectual content.
All authors read and approved the final manuscript.

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Declaration of Conflicting Interests

The authors hereby declare no conflict of interest for the research, authorship, and/or publication of this article.

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