Acute and sub acute toxicity study of an anti Diabetic Siddha Polyherbal Preparation, 
Atthippattaiyathi Kasayam.

H. Nalini Sofia¹, H. Vetha Merlin Kumari¹, ThomasM.Walter², SG Senthil Kumar³
¹ Lecturer, Department of Maruthuvam, National Institute of Siddha, Chennai, Tamil Nadu, India.
² Lecturer, Department of Pharmacology, Government Siddha Medical College, Palayamkottai, Tamil Nadu, India.
³ Siddha Physician, Herbal Medical Centre, Thanjavur, Tamil Nadu, India.

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ABSTRACT
Atthippattaiyathi kasayam (APK), a polyherbal Siddha formulation intended to use for diabetic patients, has been screened for toxic effects. For acute toxicity studies, Atthippattaiyathi kasayam was administered orally in single dose of 270 mg/kg to the mice. For sub-acute toxicity studies, different doses of Atthippattaiyathi kasayam (270, 1350 and 2700 mg/kg) were administered orally to the rats once daily for 28 days. Animals were observed for physiological and behavioral responses, mortality, food and water intake and body weight changes. All the animals were sacrificed on 29th day and changes in organ weights and histology were examined. No mortality was observed up to 270 mg/kg of Atthippattaiyathi kasayam in acute toxicity study. Daily administration of as high as 2700 mg/kg dose of Atthippattaiyathi kasayam did not result in any mortality or changes in gross behavior, body weight, weight and histology of different organs. Therefore such doses may be safe for daily administration without causing any serious side effects.

KEYWORDS: Atthippattaiyathi kasayam, Polyherbal, Siddha medicine, Diabetes mellitus, acute and sub-acute toxicity.

INTRODUCTION
Herbal medicines are popular remedies for diseases used by a vast majority of the world’s population. Herbal formulations, which have attained widespread acceptability as therapeutic agents in India, include nootropics, antidiabetics, hepatoprotective agents, and lipid-lowering agents. The pharmacological effects of many plants have been studied in various laboratories, whereas there are many limitations regarding the safety and efficacy of these preparations. Diabetes Mellitus is a chronic endocrine disorder caused by an absolute or relative lack of insulin and/or reduced insulin activity that results in hyperglycemia and abnormalities in carbohydrate, fat and protein metabolism. Diabetes has emerged as a major healthcare problem in India. Type 2 Diabetes is one of the major health problems all over the world. The management of diabetes is considered a global problem. The modern drugs including insulin and oral hypoglycemic agents control the blood sugar level as long as they are regularly administered, and also produce a number of undesirable side effects. The treatment of diabetes has been attempted with different indigenous plants and polyherbal formulations. Pre-clinical toxicity studies are essential for determining a safe dose for human trials. Atthippattaiyathi kasayam, a polyherbal antidiabetic Siddha formulation containing 22 ingredients of herbal origin that is used in traditional medicine to treat type 2 diabetes, contain both antidiabetic and antioxidant principles. Ficus recemosa (bark), Cassia fistula (bark), Cassia auriculata (bark), Salacia reticulata (bark), Madhuca longifolia (bark), Tamarindus indica (bark), Terminalia arjuna (Bark), Hemidesmus indicus (root), Amaranthus tricolor, Phyllanthus reticulates (root), Aloe barbadensis, Cyperus rotundus (root), Tinospora cordifolia (stem), Zingiber officinale (rhizome), Piper nigrum (dried fruit), Piper longum (dried fruit), Myristica fragrans (aril), Syzygium aromaticum (bud), Spermacoce hispida (root) and Ferula asafoetida (gum resin) are proven antidiabetic drugs. Therefore, an attempt was made to evaluate acute and sub-acute toxicity of a polyherbal Siddha formulation, Atthippattaiyathi kasayam in laboratory animals. The experimental protocol was approved by the Institutional Animal Ethical Committee of National Institute of Siddha, Chennai (1248/ac/09/CPCSEA/4-07/2011).

MATERIALS AND METHODS
Collection of plants:
Atthippattaiyathi kasayam formulation was prepared using Ficus recemosa (bark), Cassia fistula (bark), Cassia auriculata (bark), 

*Corresponding author.
H. Nalini sofia, Lecturer,
Department of Maruthuvam,
National Institute of Siddha, Chennai,
Tamil Nadu, India.
Preparation of aqueous extract:
The aqueous extract for acute study prepared by powdered 2.7 gm of Atthippattaiyathi kasayam formulation in 200ml of water and boiled it for some minutes to reduce 10 ml, allowing the decoction stand for 30 minutes and filtered it through paper filter. The volume of the filtered solution was increased to 10 ml with distilled water so that 1 ml of the solution was equivalent to 270 mg of starting material\[21\]. For sub-acute study, 2.7 gm, 13.5 gm and 27 gm of Atthippattaiyathi kasayam powder was added to 200ml of water separately and boils it for some minutes to reduce 10 ml, allowing the decoction stand for 30 minutes and filtering it through paper filter. The volume of the filtered solution was increased to 10 ml with distilled water so that 1 ml of the solution was equivalent to 270 mg, 1350 mg and 2700 mg of starting material respectively\[21\].

Experimental animals:
Acute and sub-acute toxicity studies were carried out in Swiss albino mice and Wistar rats, respectively. Animals were obtained from animal house, Kings Institute, Chennai and stocked at animal house, National Institute of Siddha, Chennai. All the animals were kept under standard environmental condition (27 ± 2 degree C). Adult mice (6 weeks old) of either sex weighing 20-25 gm were housed in polypropylene cages, 5 animals per cage with free access to water and standard pellet diet (Sai Meera food Pvt. Ltd, Bangalore). 6-8 weeks old Wistar albino rats of either sex weighing 150-200 gm were housed, 5 per cage in polypropylene cages with free access to food and water. The principles of laboratory animal care were followed.

Acute toxicity study:
For acute toxicity studies, 20 mice were used for the study. The mice were divided into 2 groups containing 10 animals. The animals were fasted overnight and the drug was administered orally. Group I received distilled water (vehicle for formulation) and served as the control. Group II received 270 mg/kg b.w of Atthippattaiyathi kasayam aqueous extract single dose orally. The animals were observed continuously for the first 4hrs then occasionally up to 24hrs and then daily up to 14 days, post treatment to observe for any toxic symptoms and mortality\[1\].

Sub-acute toxicity study:
For sub-acute toxicity studies, rats were divided into 4 groups of 10 animals each (5 males and 5 females). The animals were fasted overnight and the drug was administered orally. Group I received distilled water for 28 days and other 3 groups were received the test drug Atthippattaiyathi kasayam formulation at the dose of 270, 1350, 2700 mg/kg b.w., once daily for 28 days. All the rats were observed for any physiological and behavioral changes and mortality. Food and water consumption was checked daily. Body weight was recorded at the beginning and weekly intervals throughout the study\[3\].

Histopathological study:
All the animals were sacrificed on day 29 under ether anesthesia. Necropsy of all animals was carried out and the weights of the organs including liver, kidneys, brain, heart, and lungs were recorded. Tissue samples of organs from control and treated animals were preserved in 10% formalin for preparation of sections using microtome. The organs included liver, kidneys, heart, lungs and stomach of the animals were preserved and they were subjected to histopathological examination. The organ pieces (3-5 micron) were fixed in 10% formalin for 24 hours and washed in running water for 24 hours. Samples were dehydrated in tissue processor and then cleaned in benzene to remove absolute alcohol. Embedding was done by passing the cleared sample through three cups containing molten paraffin at 50 degree Celsius and then a cubical block of paraffin made by the L moulds it was followed by microtome and the slides were stained with haematoxylin–eosin stain. Stained sections of each organ were examined under light microscope at high (40x) power magnification. All the histopathological slides were prepared at Dept of Pathology, Vels University, pallavaram, Chennai.

RESULT AND DISCUSSION
No acute mortality was observed on oral administration at the dose of 270 mg/kg of Atthippattaiyathi kasayam formulation and all the animals were found to be normal during and at the end of the observation period (14 days). In the sub-acute toxicity study, there was no death in the treatment period either in control or in the treated groups. Food and water consumption did not differ significantly. There was no change in the general behavior or other physiological activities of the animals. None of the organs in the treated rats showed any significant change in weight. Histological examination of the organs did not reveal any pathological changes. These observations were similar in the male and female rats (Fig. 1-28).

The result shows that a very high oral dose was tolerated by the mice
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<td>Fig.27 After 1350mg/kg APK treatment</td>
<td>Fig.28 After 2700mg/kg APK treatment</td>
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without producing any toxicity symptoms. The ingredients present in the formulation are purely phytochemical in origin and contain alkaloids, flavonoids, glycosides, carbohydrate and amino acids. Since, a number of phytoconstituents are present in the formulation, these experiments were designed to screen for any toxic effects. Since, no toxic effects were observed, it could be inferred that the basic principle in the use of crude plant products or polyherbal preparations in traditional medicine, is that the toxic effect of one component is nullified by the protective effect of the other components, without interfering with their therapeutic properties. Daily administration of Athhippattaiyathi kasayam formulation at different doses of 270, 1350, 2700 mg/kg for 28 days was well tolerated and there was no cumulative toxicity of the drug, making it suitable for the treatment of chronic diseases like Diabetes Mellitus.

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