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**Evaluation of Physiochemical, Biochemical Properties
and Acute Toxicity Studies of *Maruthampattai kudineer***

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Abstract

In current scenario there is an increasing awareness and acceptability in the use of herbal medicines. Over 80% of the world population depends on herbal medicines and product for healthy living. Siddha is the holistic system of codified life style health care perfected thousands of years ago in Tamil speaking peninsular India. It is developed by the siddhars, the ancient supernatural spiritual saints of India. The Siddha system of medicine uses a fascinating combination of herbs, minerals, metals to promote good health and longevity. The present study was designed to investigate a polyherbal siddha formulation *Maruthampattai kudineer* (MP). The aim was to evaluate its safety and acute oral toxicity studies. The toxicity study was carried out in female wistar rats and the animals were treated with 2gms/kg bwt of *Maruthampattai kudineer* (MP) and they were observed for toxic signs for 14 days following (OECD) test guidelines 423. The physico-chemical and biochemical analysis was done to find out the presence of organic and inorganic compounds. Acute oral toxicity study showed no lethality in experimental animal. Thus, *Maruthampattai kudineer* (MP) proves to be a safe medicine.

Keywords: *Maruthampattai kudineer* (MP), siddha formulation, Physiochemical, biochemical, acute oral toxicity

Introduction

Siddha system of medicine is a time tested, ancient, and effective system of southern India. It is the holistic system of codified life style health care perfected thousands of years ago in Tamil speaking peninsular India. It is developed by the siddhars, the ancient supernatural spiritual saints of India. ¹Though herbal products have become increasingly popular throughout the world, one of the impediments in its acceptance is the lack of standard quality control profile. The quality of herbal medicine that is, the profile of the constituents in the final product has implications in efficacy and safety. ²Siddhars are the pioneers in preparing herbal, metal and mineral drugs. *Maruthampattai kudineer* is a polyherbal preparation

mentioned in the book Agathiyar 2000 and it is used in the treatment of diabetes mellitus.

According to siddha system diabetes can be correlated with madhumeagam. Madhumeagam is a chronic metabolic disorder commonly known as "Neerizhivu" characterized by increased and frequent urination, which is sweet in odour, resulting in gradual diminution of body's constitution.

India is the second most populous country in the world in the global diabetes epidemic. As per international Diabetes federation (2013), approximately 50% of Indian population (65.1 million) are affected by diabetes.

Non communicable disease are becoming more prevalent nowadays due to life style modification. Many plants and their products have been widely prescribed and used for diabetic treatment all around the world with less known mechanistic basis of their functioning. Thus, these natural products need to be evaluated scientifically for its global acceptance.

Materials and Methods

Source of raw drugs:

The required raw drugs for the preparation of *Maruthampattai kudineer* were procured from the raw drug shop, Parrys, Chennai.

Raw drugs Identification and authentication:

The ingredients were identified and were authenticated by, Medicinal Botanist at NIS, Tambaram sanatorium, Chennai.

Ingredients:

1. Maruthampattai (*Terminalia arjuna*) -350 gms
2. Navalpattai (*Syzygium cumini*) - 350 gms
3. Karuvellampattai (*Acacia nilotica*) - 350 gms
4. Athipattai (*Ficus racemosa*) - 350 gms
5. Avaraihol (*Cassia auriculata*) - 350 gms
6. Kadalalinjilpattai (*Salacia reticulata*) - 700 gms
7. Thetrankottai (*Strychnos potatorum*) - 35 gms
8. Kalipakku (*Areca catechu*) -35 gms
9. Kadukkaithol (*Terminalia chebula*) -35 gms
10. Nellivatrul (*Phyllanthus emblica*) - 35 gms
11. Thandrikaithol (*Terminalia bellirica*) - 35 gms

Preparation:

The above ingredients were ground into coarse powder

Physicochemical analysis:

Determination of pH range:

The pH of crude powder in 10 % w/v of water soluble portions of whole plant powder was determined using standard simple glass electrode pH meter.

Determination of moisture content (loss on drying):

This step was done by placing about 1.0 g of whole plant powder, in an accurately weighed moisture disc. For estimation of loss on drying, it was dried at 105°C for 3hour in an oven, cooled in a desiccator for 30 minutes and weighed without delay. The loss of weight was calculated as the content of in percent of air-dried material.

Determination of total ash:

2 g of air dried coarsely powdered drug was placed in a previously ignited (350°C for 1 hour) and tarred crucible accurately weighed. Dried material was spread in an even layer in the crucible and the material ignited by gradually increasing the heat to 550°C for 5 hours in a muffle furnace until it is white, indicating the absence of carbon. Cooled in a desiccator and weighed. Total ash content was calculated in mg per g of air-dried material.

Determination of acid insoluble ash:

The ash was washed from the crucible into 100 ml beaker using 25 ml of 2N HCl. It was then boiled for 5 min over a Bunsen burner and filtered through an ashless filter paper (Whatman No:42). The residue was washed with hot water twice, ignited to ash, cooled in desiccators and weighed. The residue was weighed and the acid insoluble ash of the drug was calculated with reference to the air dried sample of crude drug. Acid insoluble ash value is frequently necessary to evaluate the crude drugs. This ash value indicates contamination with siliceous material e.g. earth and sand. The comparison of this with the total ash value of the sample will differentiate between contaminating minerals and variations of the natural ash of the drug.

Determination of water soluble extractive value:

Determination of water soluble extractive value is used for evaluating crude drugs which are not readily estimated by other means. The extracts obtained by exhausting crude drugs are indicative of approximate measure of their chemical constituents. This method is applied to drugs which contain water soluble active constituents of crude drugs such as 65 tannins, sugars, plant acids, mucilage and glycosides. The water soluble extractive value can be used to indicate poor quality, adulteration with any unwanted material or incorrect processing of the crude drug during the process of drying and storage. About 5 g of powdered plant material was added to 50 ml of water at 80°C in a stoppered flask. It was shaken well and allowed to stand for 10 minutes. It was cooled to 15°C, 2g of kieselghur was added into it and filtered. Transferred 5 ml of the filtrate to a tarred evaporating basin and evaporated on a water bath and the residue was weighed. The percentage of water soluble extractive was calculated with reference to air dried drug.

Determination of alcohol soluble extractive value:

Alcohol is an ideal solvent for extraction of various chemicals like tannins, resins. Therefore this method is frequently employed to determine the approximate resin content of drug. Generally 90% ethyl alcohol is used for determination of alcohol soluble extracts. Alcohol soluble extracts are one of the tools for standardization of crude drug. Macerated 5 g of dried coarse powder of plant material with 100 ml of 90% ethanol in a closed flask for 24 h, shaking frequently during 6h and allowing to stand for 18h. It was filtered immediately taking precaution against loss of alcohol and 25 ml of filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105°C and weighed. The percentage of alcohol soluble extractive was calculated with reference to air dried drug.

Biochemical Analysis:

Preparation of Extract:

5ml of sample was taken in a 250ml clean beaker and added with 50ml of distilled water. Then it is boiled well for about 10 minutes. Then it was cooled and filtered in a 100ml volumetric flask and made up to 100ml with distilled water. This preparation was used for the qualitative analysis of acidic/basic radicals and biochemical constituents in it by the method of Kolkate.

Acute toxicity study:³

The Acute Oral Toxicity study was performed in accordance with OECD test guideline-423. The study was conducted on Female wistar rats. These animals were selected because of the recommended rodent species for oral studies as per followed guideline Female wistar rats, 8-12 weeks old, 140-160gm body weight were used for the study. The body weight range was within $\pm 20\%$ of the mean body weight at the time of Randomization and grouping.

Rats were kept under acclimatization for a week. Rats were randomized as control and treated group. Control group received distilled water and the treatment group was administered with *Maruthampattai kudineer* 2gms/kg bwt. was a single dose p.o.

The dose was prepared of a required concentration before dosing by dissolving, in distilled water. Administration: The test drug *Maruthampattai kudineer* was administered orally to each female wistar rats as single dose using a needle fitted on to a disposable syringe of appropriate size.

After drug administration observations were started to be recorded at the $\frac{1}{2}$ hr, 1hour, 2hours, 4 hours on day one of dosing and twice daily after that for the next 13 consecutive days. At the 14th day, sensory reactivity to stimuli of different types was conducted. Auditory stimuli responses were measured by clicker sound from approximately 30 cm to the rats; visual stimuli response were measured with the help of shining pen light 68 in the eye of rats and placing a blunt object near to the eye of rats. Response to proprioceptive stimuli was measured by placing anterior/dorsal surface of animals paw to the table edge. The responses of reactions for these three exercises were normal in animals belonging to both the controls as well as drug treatment dose groups. On day 15, the overnight fasted animals (water allowed ad libitum) were sacrificed and examine for gross pathological changes in the major internal organs.

Individual weights of animals were determined before MP administration, weekly thereafter and at 14 days. The quantity of feed was accessible based on the requirement to the group of animal housed in each cage (3 rats) and the same was record.

At the end of study period, the overnight fasted (water ad libitum) animals were anaesthetized with ketamine, the animals in control and *Maruthampattai kudineer* treated group were sacrificed on 15th day and gross pathological changes were observed in the experimental animals.

Results and Discussion

Table 1: Physicochemical properties of *Maruthampattai kudineer*

1	Colour	Brown.
2	Odour	Pleasant.
3	Taste	Astringent.
4	Consistency	Coarse powder
5	Description	Brownish powder
6	pH (10 % w/v aq. solution)	4.96
7	Loss on drying at 105°C	11.58 %
8	Total ash	16.74 %
9	Acid-insoluble ash	0.98 %
10	Water-soluble extractive	24.29 %
11	Alcohol soluble extractive	18.96 %

The product was found to be a light brown solid, with a pleasant odour and astringent taste. The pH range was 4.96. The water extract gave a maximum yield of 24.29w/v. Physicochemical parameters of the MP are shown in Table 1. The loss on drying value obtained is an indicative of amount of moisture content present in the drug. The loss on drying at 105°C in MP was found to be 11.58%. The total ash value was 16.74% and its an indicative of total amount of inorganic material. The acid insoluble ash value of MP was 0.98%. Analytical result showed that water soluble ash was found to be 24.29%. The percentage yield of alcohol and water extractive value (24.29 and 18.96) helped us in determining the amount of active constituents found in the preparation.

The acidic radicals test shows the presence of Sulphate, phosphate, carbonate. The basic radical test shows the presence of Calcium and absence of heavy metals such as Lead, Iron, Arsenic and Mercury. The Miscellaneous test shows the presence of Alkaloids, Tannic acid, Oxyquinalone, epinephrine and pyro catechol.

Results of toxicity studies:

According to the OECD guideline 423 when there is information in support of non-toxicity or low and immortality nature of the test substance, then the limit test at the dose level 2 gms/kg body weights (highest

starting dose level) was conducted. All animals were observed daily once for any abnormal clinical signs.

No mortality was observed during the total period of the study. Data obtained in this study indicated no significance physical and behavioural signs of any toxicity due to administration of *Maruthampattai kudineer* at the dose of 2gms/kg to rats. At the 14th day, all animals were observed for functional and behavioural examination. In functional and behavioural examination, home cage activity, hand held activity were observed. Home cage activities like Body position, Respiration, involuntary movement, (Clonic and Tonic), Palpebral closure, Approach response, Touch response, Pinna reflex, Sound responses, Tail pinch response were observed. Handheld activities (Reactivity and Handling), Palpebral closure, Lacrimation, Salivation, Piloerection, Papillary reflex, abdominal tone, Limb tone were observed. Functional and behavioural examination was normal in all groups. Food consumption of all treated animals was found normal as compared to control group. Body weight at weekly interval was measured to find out the effect of *Maruthampattai kudineer* on the growth rate. No significant body weight changes were observed between control and treatment group. There were no treatment related mortality in both control and treatment groups throughout the experimental period. No pathological (gross) changes were observed in the experimental animals.

Table 2 Effect of *Maruthampattai kudineer* on Body weight of Wistar Rats in acute toxicity study

Treatment	Body Weight		
	Day 1	Day 7	Day 14
Control	144±.27	154.5±0.45	162.7±0.64
MP (2000mg/kg)	149±0.32	153±0.17	160±0.75

Values are expressed as mean ± SD. Statistical significance (p) calculated by one way ANOVA followed by dunnett's. $P < 0.05$ considered as significant by comparing treated group with control group using Graph Pad Prism.

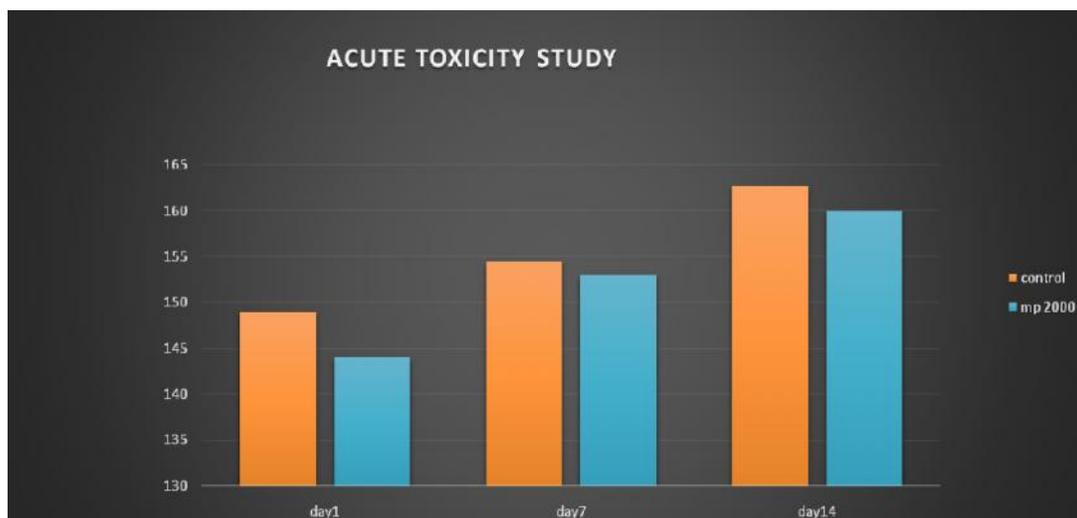


Figure 1

Conclusion

Based on the above study, the Siddha herbal preparation MP is non toxic in nature shown by acute toxic study even when it is consumed more than its therapeutic dose. In this study, we evaluated the physicochemical characteristics, biochemical and acute toxicity effects of MP. Thus it can be concluded that *Maruthampattai kudineer* is a safer drug for human consumption.

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