Scientific studies of a popular Sri Lanka indigenous therapeutic agent "Rathakalka" used in paediatric practice

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Abstract

The test drug Rathakalka, selected for these studies, is a popular Sri Lankan indigenous recipe specially used for children. A clinical study of Rathakalka recipe revealed significant changes in serum Immunoglobulins (IgG, IgM and IgA) and serum complements (C3 and C4) levels in infants and young children. Animal experiment with albino rats showed its highest anti-inflammatory activity 3 hours after induction of edema. In-vivo experiment demonstrated that Rathakalka reduced yeast induced elevation of the body temperature in rats. In-vitro experiment revealed that the recipe has anti-bacterial effect on *Staphylococcus aureus*, *Pseudomanas aeruginosa*, and *Listeria monocytogenes*. In-vivo experiment showed that the prolonged administration does not produce any toxicity changes in rabbits. Microbiological study indicated that the microbial colony counts observed in this study were within the limits acceptable by the World Health Assembly (WHA). These results scientifically evaluate that the drug samples are tested and deemed microbiologically safe and up to the microbial quality standards. These studies confirmed the presence of immune enhancing effect, anti-bacterial effect, anti-pyretic effect, anti-inflammatory effect, non toxicity, and microbiological safety in Rathakalka.

Introduction

In Sri Lanka the indigenous system of medicine has been practiced successfully since several centuries. The test drug undertaken for this study is a popular compound of Sri Lankan indigenous medicine prescribed routinely for infants by traditional physicians. It is found in Watikaprakaranaya [1] a compilation of indigenous medicine in Sri Lankan in the name of *Desadunkalka*. Another indigenous medical text called Vaidyaka Tatvadarshana [2] also carries reference of the same drug by the name of *Dvichandna kalka*. It has been included in Ayurvedic Pharmacopeia [3] published by the government of Sri Lanka in the recent past known as *Desadunkalka or Rathakalka*.

The name Desadum kalka or Dvichandna kalka implies the presence of two kinds of chandana (sandal) viz. Swetha chandna (sandalwood) and raktha chandana (red sandalwood) in the ingredients. According to the indigenous medical texts, this particular drug has been used for two main purposes, one to use it as a preventive medicament to protect infants from rakthaja roga, diseases caused by vitiated blood and the other to use it as a curative measure for fever, inflammation, respiratory diseases and some skin disease conditions.

Rakthja roga also have been named as Rathagaaya in indigenous medical system in Sri Lanka [4]. This very drug have been known as "Rathakalka" as it is used for Rathagaaya.

The ingredients of Rathakalka are:

1. *Dolichos biflorus* – Kollu (S), Horse gram (E)
2. *Glycyrrhiza glabra* – Welmee (S), Licorice (E)
3. *Acorus calamus* – Wadakaha (S), Calamus (E)
4. *Pterocarpus santalinus* – Rathandun (S), Red sandalwood (E)
5. *Santalum album* – Suduhandun (S), Sandalwood (E)
6. *Rock salt* – Sahind lunu (S)

(S – Sinhala, E – English)

Fine powder of all above herbal ingredients in equal parts and rock salt five parts are taken. After mixing, these are ground with lime (*Citrus medica*) juice to make it into form of kalka (paste).

Although this recipe Rathakalka has been used in Sri Lankan traditional medicine for many centuries, claims regarding its properties were mainly anecdotal and had not been subjected to any type of scientific confirmation. This is not only the first controlled study carried out with Rathakalka, but also pioneering study in Sri Lanka, of indigenous recipe used in traditional medicine for treatment of various disease conditions in infants and young children.

Effect of oral Rathakalka on immunoglobulin and complement levels in neonates

One of the main uses of this recipe is as a preventive medicament against various disease conditions. Therefore, it was hypothesized that the recipe may help in stimulating the immune system.

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Randomized samples of neonates were used. Test group (n=20) was given Rathakalka 250 mg for two months and control group was on placebo for two months. Serum immunoglobulins (IgG, IgA and IgM) and complements (C3 and C4) assessed in neonates from cord blood and later at the end of two months follow up by Single Radial Immunodiffusion technique. Pre-treatment levels of immunoglobulins and complements were compared with the post treatment levels.

Table 1: Effect of Rathakalka on immunoglobulins and complements

Immunoglobulin and complements	*Control mg/dl*	*Treated mg/dl*
Ig G	470	765**
Ig A	27.2	45.2**
Ig M	42.5	68.5**
C3	77.1	91.3*
C4	12.2	15.8*

** p<0.001 * p<0.01

The recipe of Rathakalka revealed significant changes in serum IgG levels in the treated group. Normally serum IgG levels decrease with the increase in age during infancy. These levels have decreased in both the groups but the physiological decrease of IgG was found less in the treated group.

Post-treatment IgA and IgM levels increased significantly (p<0.001) in the treated group. Serum C3 and C4 levels were also found enhanced significantly (p<0.01) in treated group[5].

Anti-inflammatory effect of Rathakalka

Although this recipe is being used as an anti inflammatory agent for ages, till now no scientific study has been reported. The objective of this study was to expose the recipe to animal experimentation to evaluate its anti-inflammatory effects.

This experiment was carried out using Sprague Dawley albino rats (250 - 275g). Three groups were used (n=6/group). The rats were orally administered with Rathakalka – 312.5 mg/animal in 200 ml of distilled water (treated), Iburprofen – 21.4 mg/animal in 2 ml of distilled water (reference) and 2 ml of distilled water (control) 1 hr before induction of paw edema.

Paw edema was induced in rats using 0.05ml of 1% carageenan (s/c). The volume of hind paw was determined by the water displacement technique.

The volume of displaced water was considered equivalent to the volume of the left hind paw. The paw volume measurements were taken before and after (at 1, 2, 3, 4, 5, 24 hours) carageenan injection.

The edema at each time was calculated in relation to the volume of hind paw before carageenan injection.

Table 2: Anti inflammatory effect (edema inhibition ratio) of Rathakalka

Interval after carageenan injection	*Anti inflammatory effect /edema inhibition ratio*	
	Rathakalka	*Ibuprofen*
01 hour	46.58	36.79
02 hours	42.18	57.53
03 hours	56.84*	59.84
04 hours	42.47	61.40**
05 hours	32.89	64.17
24 hours	33.51	15.02

* p<0.05 **p<0.01

Rathakalka showed its highest anti-inflammatory activity at 3 hrs after induction of edema (p<0.05, students' t-test) while Ibuprofen elicited its maximum activity at 5 hrs after carageenan treatment (p<0.01) [6].

Anti-pyretic effect of Rathakalka

The present study was undertaken to evaluate the ant-pyretic effects of Rakthakalka on yeast-induced fever in Wistar strain albino rats. Animals were randomly divided in to five groups of six each. After measuring the basal rectal temperature, animals were injected subcutaneously with 10 ml/kg body weight of 15% w/v yeast, suspended in 0.5% w/v methylcellulose solution.

Rathakalka was administrated orally 19 hours after yeast injection, at the dose of 100, 200 and 300 mg/kg body weight to 3 groups of animals respectively.

Distilled water (2 ml/animal) administrated orally to the control group. The fifth group of rats received standard drug, Paracetamol 150 mg/kg body weight orally.

The subcutaneous injection of yeast suspension markedly elevated the rectal temperature at the 19th hour after administration. Treatment with Rathakalka at the dose of 100, 200 and 300 mg/kg body weight decreased the rectal temperature of the rats in dose dependent manner.

Table 3: Anti-pyretic effect of Rathakalka

Treatment	*Rectal temperature (°C) before and after yeast injection at*				
	0 h	19 hrs	20 hrs	21 hrs	22 hrs
Control 2ml dw/animal	37.3	39.6	39.6	39.5	39.5
Paracetamol 150mg/kg^{-1}	37.2	39.7	38.3*	38.3*	37.7*
Rathakalka 100 mg/kg^{-1}	37.3	39.6	38.7*	38.3*	38.2*
Rathakalka 200 mg/kg^{-1}	37.4	39.8	38.8*	38.3*	38.2*
Rathakalka 300 mg/kg^{-1}	37.1	39.6	38.6*	38.2*	38.0*

* $p < 0.01$, as compared to control values for the corresponding hour

The anti-pyretic effect started as early as one hour after drug administration, and effect was maintained for 4 hours. The standard drug Paracetamol at the dose of 150 mg/kg body weight reduced the yeast-induced elevation of body temperature.

The results obtained from both, standard drug Paracetamol and Rathakalka treated group were compared with the control group. A significant reduction ($p<0.01$) in the yeast-elevated rectal temperature was observed.

Rathakalka has the capacity to reduce yeast induced elevation of the body temperature in rats. Results of this study may justify more scientific testing of the use of Rathakalka as an anti-pyretic agent in humans [7].

Anti-bacterial effects of Rathakalka

This particular drug is being used as a curative measure for fever, some skin diseases and respiratory disorders. One of the main causes for above disease conditions is bacterial infection.

Although individual studies of some of the ingredients in Rathakalka have been reported to have anti bacterial activity, the whole compound itself has not yet been proved to have this effect. Based on this foundation it was hypothesized that Rathakalka may have antibacterial effect as whole.

Hence the objective of this study was to evaluate the antibacterial effect of Rathakalka.

0.1gm of Rathakalka was dissolved in 5 ml of sterile distilled water and filter sterilized using Hemmings filter. This sterile soluble extract of Rakthakalka was used in this study. Anti-bacterial assay was performed using cylinder plate method in nutrient agar and incubated for 48 hours at room temperature. Bacterial species used were *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and *Listeria monoytogenes.*

Table 4: Anti-bacterial effects of Rathakalka

Bacterial species	*Diameter of inhibition zones*
Staphylococcus aureus	2.40 cm
Pseudomonas aeruginosa	1.75 cm
Listeria monocytogenes	1.40 cm
Eschichia coli	

A clear inhibition zone of bacterial lawns were observed repeatedly in all plates except *E. coli* indicating that the water extract of Rathakalka has anti-bacterial effect on *Staphylococcus aureus, Psedomonas aeruginosa* and *Listeria monoytogenes* [8].

Toxicity study

In view of extensive use of Rathakalka in neonates over a long period it is argued that toxicity test in animals are superfluous. On the other hand there is growing awareness among scientists and general public of the ill effects of drugs. The objective of this study was to expose the recipe of Rathakalka to animal experimentation to determine the presence of toxic effects if any.

Thirty Belgium strain white rabbits (weight 900-1000 g) were randomized into three groups of ten, each group comprising an equal number of male and female rabbits. One group served as control and received 15ml/Kg body weight distilled water and the other two groups recived Rathakalka at a dose of 1 gm and 3 gm/Kg body weight respectively for 60 days. The animals were observed for any signs of toxicity throughout the period of the experiment. On day 61 blood and urine samples collected and the animals were sacrificed and observed for any macroscopical changes and histo-pathological examination of target organs.

No mortality was observed during the course of experiment. Urinalysis and haematological parameters were within the normal range. No observable gross abnormalities that could be attributed to drug toxicity were noticed in the treated group. Histo-pathological examination of the target organs revealed total normalcy. The study concluded that the recipe of Rathakalka does not show any evidence of toxicity when tested on rabbits. It is probable that the same absence of toxicity would be observed when Rathakalka is used as a medication in infants and children [9].

Total viable count and specific micro organisms in Rathakalka

Basic ingredients of Rathakalka are mainly plant origin. All such materials contain a natural inherent microbial flora and also may contain added contaminant during processing, preparation, and storage.

Considering these facts the World Health Assembly in its resolutions WHA - 31:33, 40:33, and 42:43 has emphasized the need to ensure the microbial quality standard of medicinal plant products by using modern techniques and applying suitable standards [10].

The main objective of this study was to enumerate the total viable count of bacteria and the specific micro organisms such as *E-coli* and *Salmonella*.

Fifteen market samples of different manufactures were studied. Three different samples of each manufacturers with different manufacturing dates were selected for the study. Nutrient agar and potato dextrose agar were used as common culture media for bacteria and fungi.

Routine sterilization process was followed to sterile the culture media and glass ware. 1.0 gram of Rathakalka was dissolved in 10 ml of sterile distilled water and three dilutions of 10^{-1}, 10^{-2}, 10^{-3} were made using this solution. 0.1 ml of this solution was used to study the microbial load. Pour plate technique and spread plate technique were used on nutrient agar and potato dextrose agar respectively. Microbial count on nutrient agar and potato dextrose agar were taken after 24 hours and 72 hours. It was assumed that each colony was formed by a single organism. Coliform test was done with single strength MacConkey broth by using probable number technique.

Salmonella was performed according to the international standard. The same procedures were repeated three times to confirm the colony count and the specific micro organisms.

The results obtained in this study indicate the presence of bacteria and fungi in this preparation. None of the drug samples was positive for Coliforms or *Salmonella*. According to the limits adapted from the provisional guide lines established by the World Health Assembly, the microbial colony counts observed in this study were within the limits acceptable by the W.H.A. These results were statistically analyzed by using t-test.

Mean of the colony count is not significant at 0.05. There is no difference of standard mean of the colony count and sample colony count. These results scientifically evaluate that the drug samples tested microbiologically safe and up to the microbial quality standard [11].

Comments

These studies have been conclusively demonstrated for the first time in Sri Lanka, the presence of immune enhancing effect, anti-bacterial effect, anti-pyretic effect, anti-inflammatory effect, non toxicity, and microbiological safety in an indigenous recipe Rathakalka, and thus help to establish the value of traditional medicines in the therapy of infantile diseases.

Sri Lankan traditional physicians have used this recipe of Rathakalka on the basis of their own indigenous knowledge and clinical experience but not on the findings of any scientific research. Therefore, the results of these preliminary studies justify scientifically the use of indigenous recipe of Rathakalka in infants and young children by traditional Ayurvedic practitioners in Sri Lanka during the last few centuries.

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