

Comparative Double Blind Trial in Anthelmintic Efficacy Between Mebendazole and *Areca catechu* L.

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Abstract: This study was carried out in Mahasarakham Primary Healthcare Centre, Mahasarakham province in the area of Northeastern of Thailand. The experiment was randomized control trial comparative clinical study in order to examine the efficacy of *Areca catechu* Linn. and Mebendazole in the treatment of anthelmintic activity of mixed worms infection in human. The experimental group consisted of 10 patients and 5 patients for control group with inclusion and exclusion criteria which were screened by the selection of mixed worm infection symptoms samples. The investigation and extraction of worm Eggs Per Gram of patient feces method (EPG) were performed with Ether Formalin Sedimentation test. The percentage of reduction of Eggs Per Gram (EPG) of patient feces were collected, counted and confirmed by parasitologist and the clinical efficacy was investigated by the physician and the pharmacist. The % EPG data were collected before and after the treatment with *Areca catechu* Linn. and with placebo. The result showed and confirmed by Wilcoxon Signed Ranks test and also confirmed Kruskal Wallis Method which showed that Mebendazole had higher in anthelmintic activity than *Areca catechu* Linn. The efficacy in anthelmintic activity in % reduction of worm eggs per gram (in patient feces) by Mebendazole treatment was 87.50 and % reduction of eggs per gram by *Areca catechu* Linn. was 68.12%. The suggestion of this study should increase the number of samples of worm infected patients which the samples can be identified with the specification helminthes genus and species in order to obtain more accurate by the treatment *Areca catechu* Linn. and Mebendazole. The future study can be carried out in various dosage forms in order to obtain the maximum efficacy in pharmacokinetic and pharmacological activity.

Key words: Efficacy, Mebendazole, *Areca catechu* Linn., egg per gram, pharmacist, Thailand

INTRODUCTION

In the tropical countries such as Thailand researchers have a high incidence rate of human parasitic infection which can be very harmful to humans. The initial stage of parasitic infection such as nematode, tapeworm and hookworm does not have sudden severe harmful effects to the human body. The symptoms will develop gradually and slowly which can destroy the internal organs. Some parasites can withdraw blood from human intestines or other internal cell wall organs and also nutrients from human bodies. The patients will slowly lose their weight and turn pale. The patients will also possess a lack of cognitive thinking and less intellect.

Over 32 countries faced the problems of clean food consumption and clean drinking tap water management supplied to the main population. There are >12 million people who suffer from helminthes infection. WHO

(World Health Organization) has considered the parasitic problem as one of the major health communicable disease issues in the world. In Thailand the Ministry of Health has launched the project in order to decrease the incidence of food borne diseases, especially parasitic diseases which occurred in people who have less exposed to sanitation of food and water intake. Normally these patients will contact the parasites through food intake, water intake and skin transmission. One cause is the style of Thai Northeastern food preparation which is involved undercooked foods. The villagers like to consume raw food from fish, pork, beef which promote worm diseases. Another reason is the polluted local drinking water that the people consume on a daily basis from the river which was not clean and was contaminated with bacteria, worms and viruses. WHO is responsible for the record of 133 million of infected worm diseases patients throughout the world. The indication showed that the numbers of

infected patients were under the age <5 years mostly. Since, 2006-2011 the incident rate of parasitic human diseases was decreased gradually from 1,051,447-800,000 patients. The worms which were discovered in Thailand were multi cellular and one structure. Helminthes live in human body and also in animal body. They work as possible parasitic condition consuming the nutrients and blood from the body. There are many species of parasites which live in soil, weed and water in the fresh of terrestrial, in aquatic, in amphibians, etc. Helminthes can transmit through oral skin absorption and even through placenta movement from mother to baby. Helminthes are tapeworm, Strongyloides, liver flukes, lung flukes and some may be travel through skin such as Trichinosis.

The patients who was infected may show the symptoms as more food intakes, weight loss, diarrhea, irritable colon, bloating, body pain and blurred vision. The patients should go to see the physician in order to have stool examination test for worm eggs per gram of faeces by any method such as Kato Katz Method or Ether-Formalin Sedimentation Test Method. Some patients have shown the severe symptoms which cause paralysis or death. Types, size, volumes and location of helminthes and also the duration of infection are important for indicating the severity of infection. Some patients exhibit signs of jaundice, liver cirrhosis and result in liver carcinoma.

The side effect of antihelminthic medicines may cause stomachache, diarrhea and irritable colon. Some also cause nausea and vomiting. The research team had introduced in the treatment of helminthes and compared the efficacy among Mebendazole (500 mg) and *Areca catechu* Linn. Mebendazole 500 mg is the drug of choice in treatment of helminthes infection in this research and considered as modern medicine. Dosage form is chewable tablet, 500 mg and taken as once daily for 3 days continuously.

Areca catechu Linn. was prepared for herbal medicine in the treatment of mixed antihelminthes infection. Dosage was 60-90 mg in dried powder capsules and taken by mixing with syrup water as once daily for 3 days continuously. All medicine were tested to meet the standardization quality with free from bacterial or foreign contamination, free from steroidal substance and free from pesticide from the Department of Oriental Medicine, Rungsit University, Thailand.

LITERATURE REVIEW

Malinee found that the comparative study among *Piper nigrum* Linn., *Areca catechu* Linn. and Thai Depree

in antihelminthic efficacy comparison in Thailand were used in treatments of mixed infections. The dosages used were 6.5, 45.5 and 6.5 g, respectively and the egg reduction rate were 80-90%.

Fabibi studied the vermifugal herbs in animals which were found in Nigeria. In the year of 2000, WHO had the health policy of promoting herbal medicinal replacements in order to supplement modern medicines which the efficacy were 77% effective.

Carsten Flohr showed the results of randomized double blind experimental trial in efficacy between Mebendazole (500) mg and Albendazole showed no difference. If there was the increase of 3 times dosage of both drugs the result showed 75% in efficacy of mebendazole and 88% in Albendazole. The experimental research was carried on and confirmed by parasitologist, pharmacists, physician and nurses. The selection of the patients was considered at the early stage of mixed worm infection diseases. Sardsaengjun and Jutiviboonsuk (2010) studied the effect of temperature and duration time on polyphenols extract of *Areca catechu* Linn. Seeds which was found that the study aimed to explore the effect of temperature and duration time on polyphenols content from extracts of areca seeds (*Areca catechu* Linn.). The extractions were performed at 3 different temperatures (60, 80 and 100°C) and 4 different duration times (15, 30, 45 and 60 min). It was found that temperature and duration time of extraction had effect on % yield (% w/w) of crude extract and the quantity (% w/w) of total polyphenols content by areca seed powder. The % yield of crude extract increased when temperature and duration time of extraction increased. The optimal extraction condition to obtain the highest amount of the crude extract (9.65±0.35%) was at 100°C for 45 min.

However, the optimal condition of extraction for obtaining the highest % w/w total polyphenols content was at 80°C for 45 min. The highest % w/w total polyphenols content equivalent to catechin was 2.76±0.19%. But this condition did not give the highest % w/w yield (4.33±0.26%). The optimal condition for the highest crude extract (% w/w) resulted in more impurities than did the optimal condition for the highest total polyphenols content. Temperature and duration time had effect on total polyphenols extraction. The optimal condition to achieve the highest % w/w total polyphenols content was 80°C for 45 min (Sardsaengjun and Jutiviboonsuk, 2010).

Karphom *et al.* (2009) studied the antimicrobial of Betel nut (*Areca catechu* Linn.) seed extracts which was found that seeds of betel nuts grown in Nakornchaisri, Nakombathom province and Banpaew and Samuthprakarn province which had round shaped, immature and mature

were extracted with 4 different solvents. They were water, 95% alcohol, acetone and ethylacetate. These crude extracts were tested for ability to inhibit the growth (MIC) of the pathogenic bacteria; *Bacillus cereus* ATCC 1729, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 13811. It was found that the components in the betel nut seeds were best dissolved in water as the water extraction also yielded the highest amount. It was found that different location affected the % yield and ability to inhibit the test microorganisms. The betel nut seeds from Nakornchaisri provided higher extract yield and better ability to inhibit the test organisms. These extracts were able to inhibit both gram positive and negative bacteria but prefer to inhibit gram positive while the water and acetone extracts showed the higher inhibition activities. The Minimum Inhibitory Concentration (MIC) of betel nut extracts on all four test organisms were about 0.78 mg mL^{-1} when tested with paper disc diffusion technique (Kaphrom *et al.*, 2009).

Jaiswal *et al.* (2011) studied the *Areca catechu* L.: a valuable herbal medicine against different health problem which was found that *Areca catechu* is a species of plam grown mainly in Asian countries for seed crop. The chemical constituents of *A. catechu* have been investigated for anti-nematodal/helmintic, antioxidant, anti-venom, modulation of phagocytosis, effect on sperm motility/catecholamine release as immune suppression, management of psychiatric disorder and Alzheimer's disease recent studies have revealed strong molluscidal activity of *A. catechu* against harmful sanils for the control of fascioliasis.

Despite its laboratory studies on nemafocidal/helmintic/molluscidal activity arecoline is the major constituents of *A. catechu* for most of their biological effect. *A. catechu* deserves more attention by scientific community and health experts to explore its full range of benefits in welfare of mankind. Adverse effects of oral ingestion of *A. catechu* causing Oral Submucous Fibrosis (OSF), oral submucous cell carcinoma should be taken inconsideration while their use is recommended in solving different health problem (Jaiswal *et al.*, 2011).

Paul and Zaman (1975) studied the a trial of Mebendazole in Trichuriasis (whipworm) infestation in Singapore children which was found that 10 cases of Trichuris infestation in children are described presenting with malaise pica, diarrhea, prolapsed of the rectum, anaemia and infection. All children suffered considerably in health by the infection aggravated by protein caloric malnutrition and anaemia. Malay children were mainly affected living in the poor rural areas with poor sanitation. Mebendazole has proved to be effective in the treatment

of Trichuriasis in children. Administered orally in tablets it is well tolerated in doses of 100 mg daily for 3 days. Pre-ova count and post-ova count of the stools while on the drug revealed a reduction rate of almost 100% in 2 weeks after administration. No side effects were observed with the drug (Paul and Zaman, 1975).

Albonico *et al.* (2003) studied the efficacy of mebendazole and levamisole alone or in combination against intestinal nematode infections after repeated targeted mebendazole treatment in Zanzibar which was found that the overall efficacy of mebendazole against hookworm infections after periodic chemotherapy is reduced. The efficacy of benzimidazoles in chemotherapy based control programmes should be monitored closely. Combined treatment with mebendazole and levamisole may be useful as a tool to delay the development of benzimidazole resistance.

Kafle *et al.* (2011) studied antifertility effect of *Areca catechu* in male Albino rats which was found that the aim of the present study was to evaluate the antifertility activity of *Areca catechu* in male albino rats. Alcoholic extract of *Areca catechu* was studied for antifertility at doses 300 and 600 mg kg^{-1} body weight. Fertility was assessed with mating test. Body weight and weight of the reproductive organs (testis and epididymis) were observed. Biochemical evaluation (total protein content and cholesterol level) and histopathological at 600 mg doses.

There was no significant change in the body weight and weight of the reproductive organs (testis and epididymes). Cholesterol in the testis was significant ($p < 0.01$) increase and total protein content was significantly ($p < 0.01$) decreased at both doses. Histology of the testis showed reduction in the number of secondary spermatocytes and spermatides. There was a reduction in the number of Leydig cells, increase in the diameter of seminiferous tubules with necrotic products and fluid collections at higher dose. Alcoholic extract of *Areca catechu* showed antifertility activity at 300 and 600 mg kg^{-1} body weight doses (Kafle *et al.*, 2011).

Dawson and Watson (1985) studied the effect of dose form on the bioavailability of Mebendazole in man which was found that four different dose forms of Mebendazole were administered to human volunteers and urine was collected and assayed for Mebendazole as an oily suspension slightly enhances the bioavailability of the drug however, Mebendazole in human is 2 amino 5 (6) [α -hydroxybenzyl] benzimidazole (IV), not 2 amino 5 (6) benzoybenzimidazole (II) as previously reported (Dawson and Watson, 1985) (Fig. 1-3).

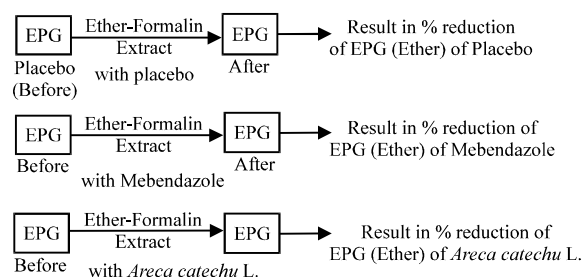


Fig. 1: Conceptual research framework; No. of patients in the experiment = 15



Fig. 2: *Areca catechu* Linn; <http://floraofsingapore.wordpress.com>



Fig. 3: Mebendazole; <http://www.stanford.edu/class/humbiol103/ParaSites2006/Enterobius/>

MATERIALS AND METHODS

This research was proceeded during September 2011 at Primary Health Care Center Mahasarakham Hospital, Mahasarakham, Thailand. The samples were screened purposively relevance to the inclusion and exclusion criteria of 16-65 years old with both genders males and females (not pregnant), non-medicated

with any of antihelminthic drugs, non-medicated with any of antibiotic, phenytoin, carbamazepine, no complication of gastrointestinal symptoms, no hepatitis or liver diseases. The collected samples were divided for 5 patients in each group for 4 groups as follows: Placebo control group, treated with MebendazoleF2 experimental group and treated with *Areca catechu* Linn. experimental group.

The Screening Method used in this experiment was ether formalin stool sedimentation examination which could be used to identify and quantify the EPG (numbers of Eggs Per Gram of feces of infected patient). The study was randomized control trial in the comparative study of the efficacy and side effects. Efficacy was comparable with the % reduction of EPG in each group with treated with Mebendazole and *Areca catechu* Linn. *Areca catechu* LinnF1.

Preparations: In this experiment we used *Areca catechu* Linn. (Betel nut) seeds in order to prepare antihelminthic medicines. Researchers have chosen the brownish white color derived from thin sliced pieces of the nuts which have a high qualitative content of Arecoline as one of the active ingredients in antihelminthic action of *Areca catechu* Linn. The betel nut seeds were weighed between 60-90 g then triturated as a very fine powder. The powder was mixed with the syrup water for oral administration. The dosage suggested was taken in syrup once daily for 3 days before breakfast.

RESULTS

The collected data were analyzed by Willcoxon Signed Ranks test. It showed that mean differences between EPG of 5 patient treated with Mebendazole (50 mg) for 3 days and EPG of patient treated with *Areca catechu* L. (60 g) for 3 days had the statistical significant differences at p-value is 0.05.

The efficacy of mebendazole (500 mg) measured by the reduction of EPG in helminthes mixed infection patient was 87.50% and the efficacy of *Areca catechu* L. was 68.12% T1. The result of this study was also confirmed by Kruskal-Wallis Statistical Method which have shows a lugher mean rank of 8th position the efficacy of mebendazole than 3rd position mean rank in efficacy of *Areca catechu* L. Therefore, it can conclude that the efficacy of Mebendazole is better than *Areca catechu* L. form thin experiment T2 (Table 1 and 2).

Table 1: Wilcoxon signed ranks test

No. of patients	Mean EPG (Placebo)	Mean EPG (Mebendazole)	Mean EPG <i>Areca catechu</i> L.	Z-value	p-value	Reduction of EPG (%)
5	463.40	65.80	-	-2.023	0.031	87.50
5	427.20	-	136.80	-2.023	0.031	68.12

Table 2: Kruskal-Wallis test

Drugs	No. of patients	Mean rank	χ^2	p-value	95% CI
Mebendazole	5	8	-	-	-
<i>Areca catechu</i> L.	5	3	12.50	0.000	0.000-0.181

CONCLUSION

This study was carried out 3 days dosages and the % EPG reduction was collected after the 4th day of the experiments in each group of samples. There may be some variations in the results after the first dosage given of each vermifugal medicine. Because some patients may have parasites presented in different locations of the human bodies such as blood stream, lung, liver, etc. Therefore, the efficacy was different in different species of helminthes. The extensive study should be recommended in various dosages and repeated dosages of 7, 14 and 20 days should be measured the end result of % reduction of Eggs Per Gram of Feces (EPG). The result would be more reliable which was suggested to increase the size of samples. The suggestion also aimed to investigate future research of specific species of helminthes for comparative efficacy and also suggest to carry out the experiment in various form of dosages in order to obtain the maximum efficacy dosage form in antihelmintic activity of *Areca Catechu* Linn.

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