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In vivo Efficacy of *Murraya paniculata* Leaf in Controlling Natural Helminthiasis in Goat

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Abstract: This study investigated the *in vivo* effect of *Murraya paniculata* leaf on gastrointestinal nematodes reduction, growth rates and haematological changes of goats. Four experimental groups (n = 6) of goats naturally acquired gastrointestinal nematodes were control (GI) (untreated), positive control (GII) (treated with albendazole, 6 mg/Kg BW) and GIII and GIV treated with 5 and 10 g/Kg BW of *M. paniculata* leaves. Fecal samples collections were performed weekly for assessment of fecal egg count. Number of nematode eggs in goat feces (egg per gram; EPG) was determined from week 0 (pre-treatment) to week 8 (post-treatment) by modified McMaster egg counting technique. EPG values of GIV goats were significantly lower than that of the controls and those receiving the albendazole ($p<0.05$) at week 3. GII goats showed highly effective at EPG reduction in 1-2 weeks after giving albendazole and then the efficiency decreased until the end of the experiment. The effect of *M. paniculata* leaves on growth rate was studied by examining goat weight on days 0, 30, 60 and 90. GIV group revealed significantly higher weight change than other groups ($p<0.05$). The feeding of *M. paniculata* leaves at different levels did not affect on haematological parameters, glucose, total protein, urea, albumin and globulin of goats. It was found that values of the haematological parameters were within the range of the standard values. Compound analysis of *M. paniculata* leaves revealed that *M. paniculata* leaves contained higher protein content than grass and the protein content was closed to the meal concentrate. This corresponds to the best growth rates of GIV goats. The results demonstrated that *M. paniculata* leaves reduce the gastrointestinal nematode eggs of goats efficiently, safely and environment-friendly.

Keywords: Orange Jessamine Leaf, Herbal Anthelmintics, Gastrointestinal Nematode, Goat, *In vivo* Test

Introduction

Goat farming is an interesting source of earnings for goat shepherd in Lopburi province, Thailand. Helminthiasis is one of the most common setbacks in production and reproductive performance of livestock (Kochapakdee, 1991). Boonkusol (2016) studied prevalence of gastrointestinal parasites in goats at different age and sex in six districts of Lopburi province and reported that strongyle-type eggs, *Trichuris* spp. eggs and coccidian oocysts were found with prevalence rates of 67.2%, 4.4% 78.8%, respectively. High infection of gastrointernal parasites were observed in goats at age of 1-3 year (57.3%) and < 1 year (36.9%). The lowest infection was > 3 years old goats (5.8%). Most of the effects caused by helminth parasitoses were unnoticed due to sub-clinical or chronic character of the diseases (Dawo and Tibo, 2005). Antibiotics use in the diseases control is no longer sustainable because of high levels of antibiotics resistance, high cost and residue problem (Waller, 2006).

The option of herbal anthelmintics has provided an important and viable alternative to control and treat these helminthic infections (Waller, 2001). Studies of anthelmintic activity of many plants are still in large scientific interest despite widespread use of chemicals in modern clinical practices as anthelmintics (Githiori *et al*., 2006; Jabbar *et al*., 2007; Eguale *et al*., 2011). *Murraya paniculata* is one of many medicinal plants used by traditional communities in Thailand. Medicinal plants are use in animal feeds as growth promoters. They reveal a major role as antioxidant, antibacterial, anthelmintic and anticoccidial without residual effects. *Murraya paniculata*, orange jessamine, leaf was reported to have *in vitro* anthelmintic activity (Boonkusol, 2017), but there was no report of vivo antihelminthic activity.

Reduction of fecal egg counts with time is an indication of *in vivo* anthelmintic activity. The therapeutic assessment was based on the estimation of production data (body weight (BW), Body Weight Gain (BWG) and helminthes eggs reduction, indicating anthelmintic activity (Burke *et al*., 2009; Deore and Khadabadi, 2010).

Since no report has addressed the *in vivo* efficacy of the *M. paniculata* leaf in controlling natural helminthosis, the present study was conducted taking the above view into consideration with the aim to assess *M. paniculata* efficacy in controlling natural helminthosis. The therapeutic assessment was based on nematodes egg reduction body weight, body weight gain and haematological parameters, indicating antihelminthic activity.

Materials and Methods

Collection of M. paniculata Leaves

Leaves of *M. paniculata* were collected from Patthana Nikhom District, Lopburi province, Thailand were selected, on the basis of their documented anthelmintic properties, according to report of Boonkusol (2017). The leaves of *M. paniculata* were harvested during the period from November 2017 to January 2018 and feed fresh to the goats.

Experimental Goats

All experimental goats were cross-bred goats (native and Anglo-Nubian breeds). Twenty-four goats (males 1-3 years of age) raised in Phatthana Nikhom districts, Lopburi province, Thailand were selected for the study expecting to be nematodes infected goats and were labeled by number tag. They were allowed to graze freely in boundary and feed concentrates as supplement. Mineral licks and water were obtainable ad libitum. Their fresh fecal samples were determined Egg count Per Gram (EPG) to confirm that the animals were naturally infected with nematodes. The goats were weighed using an overhead spring balance (0-50 kg scale), fresh fecal samples collected directly via rectum into clean, capped, air-tight plastic sample containers ready for EPG counting.

Treatments

The experimental goats were randomly divided into four groups (n = 6): GI (untreated), GII (treated orally with albendazole, 6.0 mg/Kg BW x 1 dose) and GIII and GIV treated orally with 5.0 and 10.0 g/Kg BW of *M. paniculata* leaves for 10 days. Fresh fecal samples were obtained from the goats before treatment (week 0) and 1st to 8th (week) post-treatment. The samples were transported in an ice box to laboratory for immediate analysis.

Blood collections from jugular vein of each goat were performed before the treatment (day 0) and post treatment at 30th, 60th and 90th day. At the date, blood collection was performed at 9 a.m. before meal and 3 p.m. after meal for 6 hours. Various hematological parameters (glucose, urea, total protein, albumin and globulin) were measured.

Individual live body weight of experimental goat was examined before the treatment (day 0) and post treatment at 30th, 60th and 90th day of experimental period using an overhead spring balance (0-50 kg scale).

Fecal Egg Count

The intensity of egg per gram of feces (EPG) was determined by modified Mc Master egg counting technique. Fecal suspension was prepared using one gram of fecal sample in 14 mL saturated salt solution. Samples were sieved through tea strainer and transferred into test tube. Mc Master egg counting chamber of was charged and eggs of gastrointestinal nematodes were counted under 10X magnification of light microscope. EPG was calculated as follows:

$$\text{Egg per gram (EPG)} = \text{Number of eggs in the chamber} \times 50$$

Eggs per gram of feces were recorded. Efficacy of different treatment was examined by fecal egg count reduction following the formula below:

$$Efficacy = \frac{(EPG\ pre-treatment - EPG\ post-treatment}{EPG\ pre-treatment} \times 100$$

Diets Analysis

Goat diets in this study, *M. paniculata* leaves, grass and meal concentrate, were analysed to determine the crude protein and moisture by proximate analysis (AOAC, 1990) and crude fiber by Van Soest methods (1987).

Data Analysis

Data analysis was performed using one-way analysis of variance (ANOVA), followed by post-hoc analysis (Dunnett's test) using statistical package programme. The significant difference level was P<0.05.

Results

Fecal Egg Count

Fecal egg count and efficacy of *M. paniculata* leaves on reduction of gastrointestinal nematodes eggs were presented in Table 1. Albendazole and *M. paniculata* leaves showed a general positive *in vivo* anthelmintic activity compared to untreated control.

Significant (p<0.05) reduction of EPG counts were observed in the 1st to 4th week following *M. paniculata* treated goats. On the other hand, EPG counts of untreated group (GI) were significantly (p<0.05) increased up to the 8th week.

Table 1: Egg per gram (EPG) values of the treated and control groups pre and post administration of *M. paniculata*

	Mean number of egg per gram (% Efficacy)			
	GI	GII	GIII	GIV
week 0	2,632.91[a]	2,766.26[a]	2,966.29[a]	2,798.81[a]
week 1	3,166.31[a]	865.35 (68.72)[b]	2,732.93 (7.87)[c]	2,566.23 (8.31)[c]
week 2	3,566.35[a]	631.64 (77.17)[b]	2,298.55 (22.51)[c]	1,532.62 (45.24)[d]
week 3	4,466.05[a]	1,098.48 (60.29)[b]	2,066.12 (30.35)[c]	664.94 (76.24)[d]
week 4	5,832.85[a]	2,299.27 (16.88)[b]	2,498.67 (15.76)[b]	1,564.93 (44.09)[c]
week 5	5,966.48[a]	3,065.77 (-10.83)[b]	3,032.97 (-2.25)[b]	2,397.16 (14.35)[c]
week 6	6,099.45[a]	4,433.08 (-60.26)[b]	3,933.05 (-32.59)[b]	3,097.81 (-10.68)[c]
week 7	6,599.49[a]	5,566.17 (-101.22)[b]	4,333.08 (-46.08)[c]	3,564.95 (-27.37)[d]
week 8	6,265.95[a]	6,232.89 (-125.32)[a]	4,999.33 (-68.54)[b]	4,199.60 (-50.05)[b]

[a,b,c,d] = Significant at p<0.05 in the same raw

Table 2: Effect of *M. paniculata* leaf on body weight in goat

	Mean of body weight (kg)			
	GI	GII	GIII	GIV
Day 0	20.27±1.10[a]	20.47±2.00[a]	20.90±4.77[a]	20.50±2.87[a]
Day 30	21.10±1.95[a]	22.20±1.78[ab]	22.40±5.39[b]	21.97±3.67[ab]
Day 60	21.20±1.87[a]	22.97±1.50[b]	23.60±4.85[b]	21.13±2.99[a]
Day 90	22.40 ±1.14[a]	23.67±1.22[b]	24.40±5.43[b]	24.53±2.65[b]
Change of BW (kg)	2.13±0.67[a]	3.20±0.90[a]	3.50±1.59[b]	4.03±0.67[c]
Growth rate (g/day)	23.67±7.40[a]	35.56±10.02[a]	38.88±10.67[b]	44.78±2.78[c]

The above values represent the mean ± Standard Deviation (SD)
[a,b,c] = Significant at p<0.05 in the same column

Table 3: Blood glucose and urea level before and after feeding

	Blood glucose (%mg) and urea (mmol/l) level			
	GI	GII	GIII	GIV
Glucose at before feeding	52.00±1.00[a]	50.37±0.12[a]	57.60±0.20[b]	51.58±0.68[a]
Glucose at after feeding	64.87±0.31[a]	60.63±0.15[a]	71.63±0.47[b]	60.00±1.05[a]
Glucose changes	12.87±0.95	10.26±0.06	14.03±0.55	8.42±0.45
Urea at before feeding	21.65±0.52[a]	19.93±0.06[a]	23.84±0.69[b]	26.29±0.12[b]
Urea at after feeding	23.32±0.08[a]	22.28±0.13[a]	26.13±0.45[b]	28.60±0.30[b]
Urea changes	1.67±0.58	2.35±0.09	2.29±0.48	2.31±0.25

The above values represent the mean ± Standard Deviation (SD)
[a,b] = Significant at p<0.05 in the same column of each parameter

There were significant (P<0.05) peaks efficacy for *M. paniculata* leaves at 5 g/kg BW and 10 g/kg BW. The 10 g/kg BW *M. paniculata* group produced efficacy of 76.24% at week 3 Post-Treatment (PT) followed by 5 g/kg BW group which gave 30.34% at week 3 PT while Albendazole gave 77.17% at week 2 PT. *M. paniculata* showed time and dose-dependent *in vivo* anthelmintic effect.

Body Weights

Changes in the Body Weights (BW) of the goats were recorded in Table 2. There were significant (P<0.05) BW changes of the goats treated with *M. paniculata* and Albendazole on day 90 PT compared with day 0. All groups had significant (P<0.05) increases in their mean BW by day 90 PT except the untreated group (GI).

The result showed the best growth rate of goats in GIV group. The daily growth rate and weight change rate were 44.78 g/day and 4.03 kg, which was higher than that of other goats.

Blood Parameters

The results of the effect of *M. paniculata* leaf on different hematological parameters glucose, total protein, urea, albumin and globulin in goat blood were shown in Table 3 and 4. The blood glucose levels before and after feeding of all groups were found to be within the standard level range (50.0 to 75.0%mg) (Plumb, 1999). However, glucose level of GI and GII goats were found to be higher than GIII and GIV goats. It might result from GI and GII goats were fed with meal concentrate which is lower fiber diets than plant leaves, resulting in rapid fermentation and conversion to glucose in the blood.

Table 4: Total protein, albumin and globulin level in blood of goats

	Total protein, albumin and globulin (g/l)			
	GI	GII	GIII	GIV
Total protein	67.47±0.35a	65.13±0.25a	70.50±0.46a	72.40±0.56a
Albumin	35.13±0.42a	36.20±0.36a	33.03±0.31a	32.83±0.25a
Globulin	31.93±0.15a	31.57±0.47a	31.03±0.21a	33.67±0.67a

The above values represent the mean ± Standard Deviation (SD)

Table 5: Percentage of crude protein, crude fiber and moisture of goat diets

Experimental food	Crude protein (%)	Crude fiber (%)	Moisture (%)
M. paniculata leaves	12.34	23.34	57.81
Grass	8.48	41.62	64.77
Meal concentrate	16.30	13.18	66.27

The blood urea levels before and after feeding of GIII and GIV goats were significantly higher than GI and GII goats, however, blood urea levels of all groups were within the standard level range (12.6 to 28.0 mmol/l) (Plumb, 1999). The standard level range of total protein, albumin and globulin were 64.0-75.0 g/l (zircon, 1995), 27.0-39.0 g/l and 27.0-41.0 g/l (Plumb, 1999), respectively. In this study, the total blood protein (65.13-72.40 g/l), albumin (32.83-36.20 g/l) and globulin (31.03-33.67 g/l) were in in the standard range in all four groups and there were no statistically significant differences of them among groups. Albumin and globulin values were.

Diets Analysis

Compound analysis of goat diets, *M. paniculata* leaves, grass and meal concentrate (Table 5) revealed that *M. paniculata* leaves contained higher protein content than grass and the protein content was closed to the meal concentrate. The results showed that total protein content of *M. paniculata* leaves, grass and meal concentrate were 12.34%, 8.48% and 16.30%, respectively. Fiber contents of *M. paniculata* leaves, grass and meal concentrate were 23.34%, 41.62% and 13.18%, respectively. Moisture content of *M. paniculata* leaves, grass and meal concentrate were 57.81%, 64.77% and 66.27%, respectively.

Discussion

In vivo anthelmintic results for *M. paniculata* leaves are being reported for the first time of conducting this study. The ingestion of *M. paniculata* leaves affected reduction of nematode eggs with efficacy of 76.19% (10 g/kg BW *M. paniculata* leaves). M. paniculate leaves also improved body weight of goat. It provides an alternative and natural anthelminthics for goat nematode that is environment friendly. *M. paniculata* leaves has medicinal properties in the treatment of relieving colic, diarrhea, dysentery and flatulence (Thinkhaonoi, 2009). It was found that water extracts of *M. paniculata* leaves affected killing of parasite *Stellantchasmus falcatus* and *Haplorchis taichui*. Boonkusol (2017) reported that both crude extracts from Tamarindus indica seeds and *Murraya paniculata* leaves showed 100% larval mortality after *in vitro* 12 h incubation. According to Wu *et al.* (2016) report, three flavonoids in glass-leaf extract exhibited anti-inflammatory activity in white blood cells and gastrointestinal mucosal cells, reducing the formation of nitric oxide and Interleukin-6. Phytochemicals in the two plants like flavonoids and oleanane type triterpenes may have had their independent or synergistic anthelmintic effects (Zafar *et al*., 2009).

Study of the substance in *M. paniculata* leaf showed that it contains 70 polymethoxylated flavonoids (PMFs), including 45 flavonoids, 17 flavonoids and 8 types of PMFs (Zhang *et al*., 2011). In addition, E-caryophyllene, Spathulenol and Delta-elemene were found. These substances exhibited significant anti-inflammatory property (Lv *et al*., 2013). Rodanant *et al*. (2015) reported that ethyl acetate extract of *M. paniculata* leaf contained coumarins, Murrangatin, Murrangatin acetate, Murranganonesenecionate, Micropubescin and Tetramethoxyflavone which have anti-inflammatory effect. In addition to *M. paniculata* leaves, there are also reports of important substances derived from its branches that contained 39 species of PMFs, including 24 flavonoids, 10 flavonoids and five types of PMFs glycosides and these compounds are resistant to inflammation (Zhang *et al*., 2013).

In the last decades, the application of naturally biological substances acquired from plants with medicinal activities is benefiting importance in human health. There are presently, a number of plants extracts that have been commercialized worldwide (Pyakuryal, 2012). Similarly, plants and their derivatives are being profoundly researched for efficacy against animal parasites. Several plants are currently being investigated to identify available anthelmintic agents for using in animals (Aguilar *et al*., 2008; Shai *et al*., 2009; Alonso-Díaz *et al*., 2010). Soro *et al*. (2013) also reported anthelmintic efficacy of Anogeissus leiocarpus Guill & Perr (Combretaceae) and improvement of body weight after *in vivo* treatment in sheep. *M. paniculata*, an important medicinal plant in Thailand, showed presently a significant fecal egg reduction and an increase in body weight in the *M. paniculata* treated goats. This finding could be an alternative mean for sustainable solving problems of high anthelmintic cost, resistance to anthelmintics and chemical residues in meat and milk for human consumption.

Blood glucose levels indicate the state of energy balance in the body of the animal ((Plumb, 1999). If there is enough energy consumption from food, blood glucose is in standard level. Blood urea is variable throughout the day, depending on several factors such as the protein level, protein degradation in the body proteolysis) during fasting and excess amino acids which were converted into urea (Lewis, 1957). In this study, urea levels were slightly higher than standard range (19.93 to 28.60 mmol/l). This may be due to excess consumption of diets protein. In addition, the present results demonstrated that the proportion of albumin to globulins in blood was not different among groups. The ratio of albumin to globulin is a measure of the health of animals by the protein globulin, which is involved. If higher levels of globulin are present, it is possible to diagnose primarily that animals have a pathogenic or parasitic infection. Diagnosis should be made in conjunction with other hematologic values, such as eosinophils are specific to the diagnosis of peritonitis (Shelly, 1997).

Conclusion

The feeding of *M. paniculata* leaves demonstrated significantly higher *in vivo* anthelmintic activity and change of body weight than untreated goats, without affecting on haematological parameters, glucose, total protein, urea, albumin and globulin of goats. Compound analysis of *M. paniculata* leaves revealed that *M. paniculata* leaves contained higher protein content than grass and the protein content was closed to the meal concentrate.

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Author's Contributions

Duangjai Boonkusol: Designed research study, conducted research, compiled the literary review, analyzed and interpreted the study findings, drew conclusions, contributed manuscript preparation.

Janejira Detraksa: Conducted research, analyzed and interpreted the results.

Kansuda Duangsrikaew: Conducted research, compiled the literary review.

Wuttipong Tongbai: Coordinated the implementation of research work. All authors have read and approved the manuscript.

Conflict of Interest

All authors confirm that this article is original and there is no conflict of interest in this article to declare.

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