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Effect of commercial drug nakku poochi (kolli) kudineer chooranum in *Haemonchus contortus*

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Abstract

The commercial drug, Nakku Poochi Kolli Kudineer (NPK) chooranum, contains extract of several herbs i.e. *Butea monosperma*, *Nigella sativa*, *Embelia ribes*, *Trachyspermum ammi*, *Operculina turpethum*, *Cassia augustifolia* and *Picrorhiza kurroa* and used for removal of round worms in human beings. An *in vitro* study with *H. contortus* was carried out to see the anthelmintic effect of this drug egg hatching and larval paralysis using NPK as concentration of 1, 3, 5, 10, 20, 30, 40, 50, 70 and 100 %. Contraction of uterus with drug, number of expelled eggs by female worm had significantly ($P < 0.05$) increased and expelling egg was dose dependent. Egg hatch was not affected and complete paralysis of worm was observed in 100% concentration of NPK at the end of 8 hours. Paralysis effect was also dose dependent. This study has proved that NPK has the ability to increase uterine contraction of female *H. contortus* thus, resulting in expulsion of numerous numbers of eggs than normal. This effect could be used in harvesting more eggs in quicker time for using Egg hatch assay. Further, NPK has also moderate paralytic activity of *H. contortus*.

Keywords: Nakku poochi kolli kudineer, *haemonchus contortus*, ovulation induction, egg hatch assay, neuromuscular effect

1. Introduction

Helminthiasis is considered as a major constraint in livestock productivity round the globe. Chemotherapeutics remain the corner stone for treating helminthiasis even though the development of problems, e.g. resistance, chemical residues and toxicity, increased cost, non-adaptability of drugs and non-availability in remote areas Hussain *et al* (2011) [1]. *Haemonchus contortus* is a predominant gastrointestinal parasite of sheep and goat Singh *et al* (1997) [2]. A variety of plants have been scientifically validated for their anthelmintic properties *in vitro* and *in vivo* Akhtar *et al* (2000) [3]. The commercial drug, Nakku Poochi Kolli Kudineer (NPK) chooranum, contains extract of several herbs i.e. *Butea monosperma*, *Nigella sativa*, *Embelia ribes*, *Trachyspermum ammi*, *Operculina turpethum*, *Cassia augustifolia* and *Picrorhiza kurroa* and used for removal of round worms in human beings. The present experiment was carried out to see the anthelmintic effect of NPK by *in vitro* assay egg hatch assay and paralysis assay that may reveal their anthelmintic activity to contribute towards the help of the local livestock owners and veterinarians in combating nematode infections in sheep and goats.

2. Material and Methods

2.1 Collection of *Haemonchus contortus*

Abomasum content with live worms of sheep was collected in normal saline from slaughter house, Perambur, Chennai. In the laboratory, abomasum content with live worms was transferred to the plastic tray containing normal saline. The adult male and female *Haemonchus contortus* (*H. contortus*) worms were separated from the abomasal contents. The worms were washed twice in the normal saline.

2.2 Harvesting of *Haemonchus contortus* eggs

The collected female worms were incubated in normal saline at 37°C for 2 hour for the release of eggs. After incubation, the normal saline was collected in centrifuge tube, leaving the worms. The tube was centrifuged at 2000 rpm for 5min to sediment the eggs. The supernatant was poured off and the sediment examined for the presence of eggs. The concentration of the eggs was adjusted to 50 eggs per 20µl of normal saline.

2.3 Preparation of stock and working solution of NPK

Commercial drug NPK 1g was transferred into a 500 ml beaker and 300 ml of distilled water was added and boiled the mixture till the total volume came up 30 ml to prepare. Using the stock solution, a wide range 1, 3, 5, 10, 20, 30, 40, 50 and 70% of working solution of NPK were prepared.

2.4 Ovulation of induction effect of NPK drug

The ovulation induction of NPK was performed in 24 well plates. To each well of 24 well plates 5 adult live female *Haemonchus contortus* was added. One milliliter of working solution (1, 3, 5, 10, 20, 30, 40, 50, 70 and 100%) and distilled water in control well were added. The plate was incubated at 37°C for 2 hour for release of eggs. After incubation, content of each well was collected in centrifuge tube separately for each concentration, leaving the worms. The tube was centrifuged at 2000 rpm for 5min to sediment the eggs. The supernatant was poured off and the sediment examined for the presence of eggs. Fifty microliter of sediments containing eggs was examined in microscopic slide and total number of eggs was counted.

2.5 Hatching inhibition effect of NPK drug

The hatching inhibition effect of NPK was performed in 24 well plates. To each well of 24 well plates, 20 µl of egg suspension with approximately 50 eggs was added and one thousand eight hundred eighty microliter of working solution of NPK was added to each well. One thousand eight hundred

eighty microliter distilled water was added to the control well. The tests were carried out with two replicates for each drug concentration plus control well. The plate was incubated at 25⁰ C for 48 hours. The number of larvae and unhatched eggs were counted under a binocular inverted microscope (Olympus SZ40, Japan).

2.6 Neuromuscular effect of NPK drug on *Haemonchus contortus*

The neuromuscular effect of NPK on *H. contortus* was performed in 24 well plates. To each well of 24 well plates 5 adult live female *Haemonchus contortus* was added. One milliliter of working solution (1, 3, 5, 10, 20, 30, 40, 50, 70 and 100%) and distilled water in control well were added. The plates were incubated at room temperature for 8 hour and the worms were examined for neuromuscular effect of NPK at 10 min, 30 min, 1, 2, 4, 6 and 8 hour intervals.

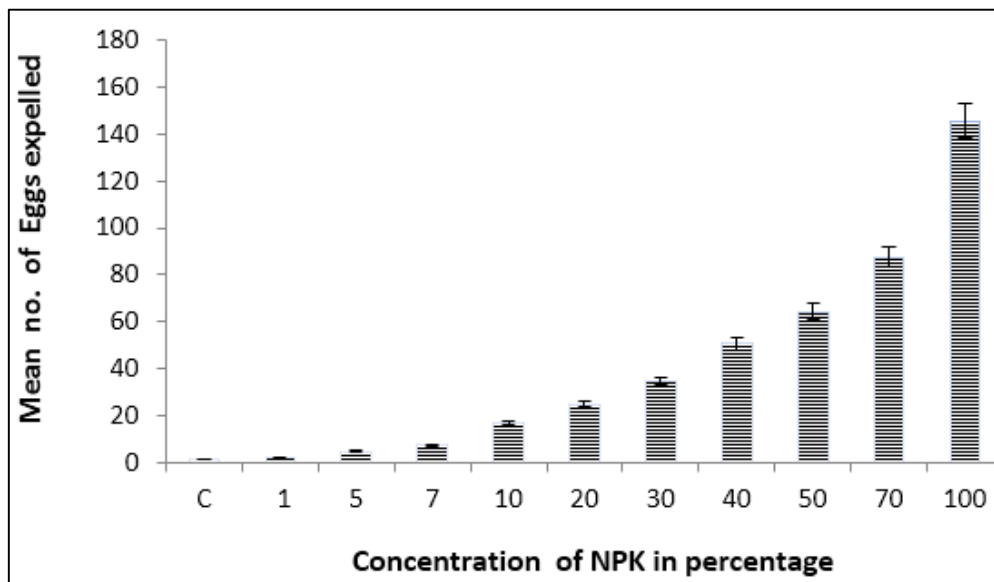
3. Results

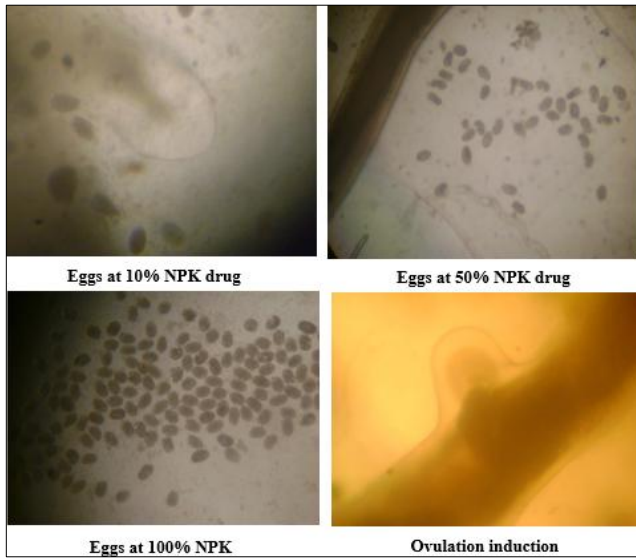
3.1 Ovulation of induction effect of NPK drug

Ovulation effect of NPK was carried out in 24 well plates in this study. The numbers of eggs were counted from each concentration after 2 hour of incubation. The mean numbers of eggs at different concentration were recorded (Table.1). The number of expelled eggs by female worm had significantly (P<0.05) increased and expelling egg was dose dependent.

Table 1: Ovulation induction effect in *Haemonchus contortus* after treatment with different concentration of NPK

Drug Concentrations (%)	Mean no. of eggs
DW	1.1±0.29
1	2.3±0.55
3	4.6±0.87
5	7.45±1.13
10	16.75±1.30
20	24.8±2.17
30	34.75±2.56
40	50.65±2.78
50	64.4±2.34
70	87.6±2.89
100	145.65±10.96





3.2 Hatching Inhibition effect of NPK

The series of egg hatch assay were performed for different concentration of NPK. The mean number of larvae hatched from eggs was recorded (Table 2.0). The number of expelled eggs by female worm had significantly ($P < 0.05$) increased and hatching egg was dose dependent.

Table 1: Hatching Effect OF NPK ON *Haemonchus contortus*

Drug Concentrations (%)	Mean no of larvae
Control	43.5±1.07
1	39.0±0.87
3	34.5±1.55
5	28.5±1.88
10	21.9±1.20
20	16.5±1.70
30	7.1±1.15
40	0.85±0.31
50	0.14±0.09
70	0.071±.007
100	0.0±.00

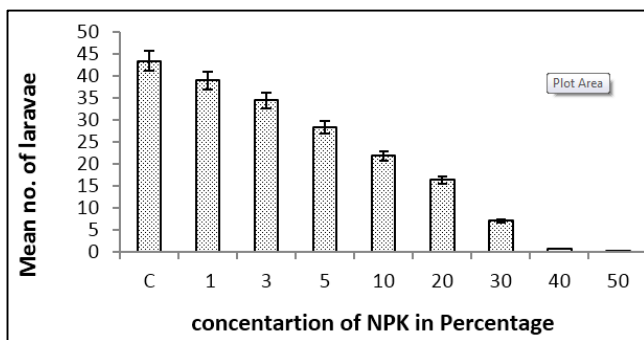


Fig 2: Hatching Effect of NPK on *Haemonchus contortus*

3.3 Neuromuscular effect of NPK drug on *Haemonchus contortus*

Neuromuscular effect on NPK on adult *Haemonchus contortus* with and without NPK drug was carried out and observed at 10 min, 30 min, 1, 2, 4, 6, and 8 hours intervals and it was found that at end of 8 hour all adult *Haemonchus contortus* worms were paralyzed at concentration of 30 to 100 % (Table 3.0)

Table 3: Neuromuscular effect of NPK (No. of paralyzed, N =5)

Con.	Control	1	3	5	10	20	30	40	50	70	100
10 min	0	0	0	0	0	0	0	0	0	0	0
30 min	0	0	0	0	0	0	0	0	0	0	0
1 hour	0	0	0	0	0	0	0	0	0	0	0
2 hour	0	0	0	0	0	0	0	0	0	0	1
4 hour	0	0	0	0	0	0	0	0	1	2	3
6 hour	0	0	0	0	0	1	5	2	3	4	4
8 hour	0	0	0	1	3	4	5	5	5	5	5

Table 3: Percentage neuromuscular effect on larvae of *Haemonchus* after treatment with different concentration of NPK (no. of paralyzed, n =5)

Con.	Cotrol	1	3	5	10	20	30	40	50	70	100
10 min	0	0	0	0	0	0	0	0	0	0	0
30 min	0	0	0	0	0	0	0	0	0	0	0
1 hour	0	0	0	0	0	0	0	0	0	0	0
2 hour	0	0	0	0	0	0	0	0	0	0	20
4 hour	0	0	0	0	0	0	0	0	20	40	60
6 hour	0	0	0	0	0	20	20	40	60	80	80
8 hour	0	0	0	20	60	80	100	100	100	100	100

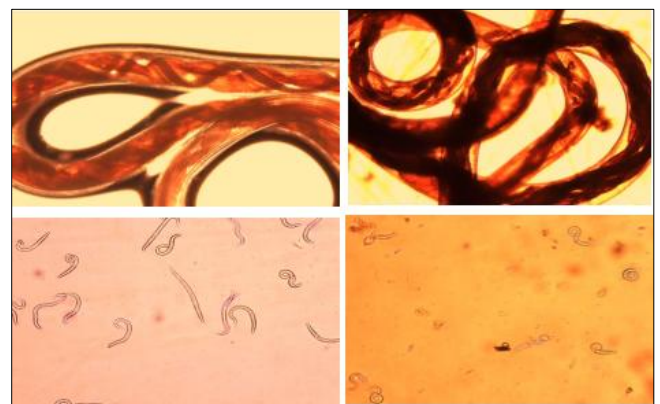


Fig: Spastic Paralysis effect of NPK on adult and larvae of *Haemonchus contortus*

4. Conclusion

The present experiment was carried out to see the anthelmintic effect of a commercial drug, NPK on gastrointestinal nematode *Haemonchus contortus* by *in vitro* assay egg hatch assay and paralysis assay that may reveal their anthelmintic activity to contribute towards the help of the local livestock owners and veterinarians in combating nematode infections in sheep and goats.

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6. References

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