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ORIGINAL ARTICLE

In vivo Evaluation of Antimicrobial, Antipyretic, Analgesic, and Anti-Inflammatory Activities of *Nilavembu Kudineer* Capsule in Comparison with Siddha Classical *Nilavembu Kudineer*

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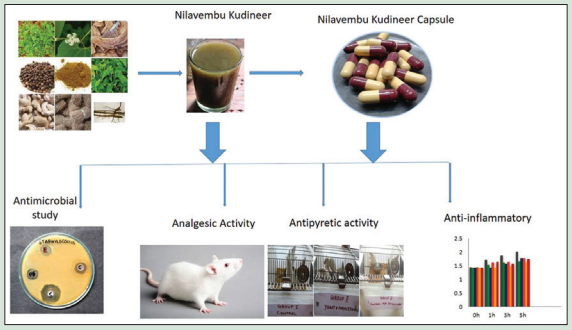
ABSTRACT

Background: The classical Siddha formulation *Nilavembu Kudineer* (NVK) is more effective in treating fever, infection, pain, and inflammation, but it is a liquid, is bitter in taste, is in a non-palatable form, and hence it was converted into a portable and palatable NVK capsule with increased shelf-life to comply with the needs of patients. The present study aimed to evaluate the effectiveness of the NVK capsule in comparison with the classical NVK by using animal models. **Materials and Methods:** NVK and NVK capsules were processed as per the standard operating procedures, and the extracts were prepared for oral administration. The antibacterial and antifungal activities of the drug in comparative assay with the standards – ciprofloxacin and fluconazole – were evaluated by the agar diffusion method. Analgesic activity of NVK and NVK Capsule was studied in Swiss albino mice of either sex (n=4), compared with positive control group; Antipyretic and antiinflammatory activity was studied in Wistar rats of either sex (n=4), compared with the positive control of Paracetamol and Indomethacin respectively.. **Results:** The zone of inhibition in antimicrobial assay revealed that NVK capsule is more effective than the extract of NVK. The NVK capsule at 200 mg/kg has equal and consistent efficacy ($P < 0.01$) to reduce pyrexia compared to paracetamol 150 mg/kg at 1–5 h. Furthermore NVK capsule at 400 mg/kg showed a statistically significant analgesic effect ($P < 0.01$) and higher level of inhibition of inflammation in comparison with NVK and indomethacin ($P < 0.05$). **Conclusion:** The study concludes that NVK capsule has very effective antimicrobial, analgesic, antipyretic, and anti-inflammatory activities in comparison with classical NVK.

Key words: Anti-inflammatory, antimicrobial, antipyretic, *Nilavembu Kudineer*, Siddha

SUMMARY

The classical Siddha formulation *Nilavembu Kudineer* (NVK) is in liquid form, bitter in taste and of short shelf-life and hence it was converted into a portable and palatable form of NVK Capsule with increased shelf-life. The aim of this study is to evaluate the effectiveness of *Nilavembu Kudineer* Capsule in comparison with classical *Nilavembu Kudineer* by using animal models. The study concludes that the portable and palatable form of *Nilavembu Kudineer* Capsule is more effective while comparing with the extract of *Nilavembu Kudineer*.



Abbreviations Used: NVK- *Nilavembu Kudineer,* EENKC- Ethanolic extract of *Nilavembu Kudineer Choornam*, CHIKV – Chikungunya Virus, DENV-2 –Dengue Virus, SOP- Standard Operating Procedures, IAEC- Institutional Animal Ethical Committee, CPCSEA – Committee for the purpose of Control and Supervision of Experiments on Animals, VC- Volume of Control, VT- Volume of Treated animals, WC- Weight of Control, WT- Weight of Treated animals, COX- Cyclooxygenase, PGE2- Prostaglandin E, SEM- Standard Error of Mean SE- Standard Error.

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INTRODUCTION

Nilavembu Kudineer (NVK) is one of the well-known classical multi-herbal Siddha formulations useful in the prevention and control of many vector-borne diseases. An investigation using the ethanolic extract of *Nilavembu Kudineer choornam* (EENKC) in inflammation, pain, and pyrexia of animal models proved its antipyretic, anti-inflammatory, and analgesic activities.[1] The oral administration of EENKC at the dose 2000 mg/kg did not exhibit any signs of toxicity up to 14 days and no animals died.[1] NVK is being commonly used for treating viral fever, dengue, chikungunya, and postfever sicknesses such as muscular and joint pains, malaise, and anorexia. *In vitro* evaluation revealed that NVK provides protection against CHIKV and DENV-2 during active infection and helps to prevent viral infection in the cells.[2]

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Health-care programs for the prevention and control of certain viral fevers such as dengue and chikungunya include the distribution of NVK in prevalent areas. An interventional cohort study in a dengue-prevalent area by using NVK showed that it is effective in reducing the clinical manifestations of dengue-fever, malaise, body pain, headache, cough, and joint pain.[3] The study revealed that the intake of NVK by normal controls on the preventive aspect can improve health status. It was concluded that NVK can impede the outbreak of dengue-like illness in endemic areas.[3]

NVK is the compound formulation comprising of nine herbal ingredients, which is bitter in taste and disagreeable to the children and/or certain population, thereby reporting the inconvenience in drug intake. Moreover, the decoction should be prepared at the time of intake because of the short shelf-life of drug, i.e., 3 h.[4] Processing of the drug as per the standard operating procedures (SOP) in all the time of dosage is of practical difficulty to the patients. Indecorous processing of drug or improper intake can reduce the effectiveness of drug NVK.

These kinds of issues concerning the palatability, portability, and shelf-life of the drug can be resolved with other dosage forms of drug NVK. Hence, the drug is converted into the new dosage form–NVK capsule, i.e., the classical decoction is concentrated and converted into the minute powder form without the exposure to overheating or water absorbents. NVK capsule is filled with minuscule powder of waterless fine molecules and hence the shelf-life is increased. The analysis of physicochemical parameters and high-performance thin layer chromatography profile of NVK capsule showed very good stability for >1 year.

The rationale of this study is to compare the efficacy of the NVK capsule with the Siddha classical NVK. The objective is to evaluate the antimicrobial, antipyretic, analgesic, and anti-inflammatory activities of different dosage forms of *Nilavembu Kudineer* in comparison with standards designed in animal model.

MATERIALS AND METHODS

Processing of the drug

The NVK and NVK capsules were processed in the Siddha Regional Research Institute (SRRI), Thiruvananthapuram, according to the SOP mentioned below. The ingredients of the NVK formulation are Nilavembu (*Andrographis paniculata*), Vettiver *(Vetiveria zizanioidus),* Vilamichan ver *(Plectranthus vettiveroides),* Santhana Thool *(Santalum album),* Peyippudal *(Trichosanthus cucumerina),* Koraikilangu *(Cyperus rotundus),* Cukku *(Zingiber officinale),* Milagu *(Piper nigrum),* and Parpadagam *(Mollugo cerviana).*[4]

The equal ratio of the above-said ingredients was collected and authenticated by the Pharmacognosy Department of SRRI, Thiruvananthapuram, and purified according to the standard procedures and pulverized into the coarse powder as *Nilavembu Kudineer Chooranam* (NVK). The 100 g of powder was mixed with 2400 ml of water, boiled well, and concentrated to 1/4th and filtered off as per the SOPs. The filtrate was lyophilized, air dried, and powdered using mortar and pestle into minute form without exposure to overheating or water absorbents. The above-said powder was mixed with talcum powder in the ratio of 7:3 in order to alter the sticky form and then filled in the empty capsule to make NVK capsule, i.e., 500 mg capsule contains 350 mg NVK powder and 150 mg talcum powder.

Extraction of *Nilavembu Kudineer* and *Nilavembu Kudineer* capsules for *in vivo* study

10 g of NVK powder was mixed with 100 ml of hydro ethanol (98% ethanol; ethanol:water ~ 98:2), and it was kept in shaking condition in a shaker at 200 rpm for 10 h at 37°C, and then the content was filtered with Whatman Number 1 filter papers and concentrated to dryness by evaporation.

Similarly, the minute powder in the NVK capsule 10 g was mixed with 100 ml of hydro ethanol (98% ethanol; ethanol:water ~ 98:2), and the extract was prepared for use in the experiment. The percentage yield was calculated using the formula below and the extract was stored in a refrigerator at 15°C until the time of use.

% yield = weight of extract material/weight of original plant material used × 100.

Micro-organisms

Seven bacterial strains and two fungal strains used in this study are the clinical isolates obtained and authenticated by the Department of Microbiology, P.S.G. Hospitals, Coimbatore. The microbial species were then subcultured for the study. The bacteria used were *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus epidermis, Pseudomonas aeruginosa, Shigella flexneri,* and *Proteus vulgaris.* The fungal strains used were *Aspergillus niger* and *Aspergillus flavus.* The pure cultures of different pathogens were grown overnight in sterile nutrient broth and incubated at 37°C for 24 h.

Animals

Healthy Swiss albino mice, *Mus musculus* (20 ± 5 g), were used for the study of anti-pyretic activity. Albino Wistar rats of either sex weighing 190 ± 10 g were used in the study of anti-inflammatory and analgesic activities. The animals were kept in polypropylene cages with sawdust bedding and maintained in laboratory conditions. Standard pellets were given as diet and water was provided *ad libitum*. The animals were acclimatized to laboratory condition for about 1 week before the commencement of the experiment.

The experiments were performed after the approval from the Institutional Animal Ethical Committee (IAEC-Resolution No. 13, 31-7-2010 dated 30-01-2019 in Avinashilingam Institute), and the procedures were carried out in accordance with CPCSEA guidelines.

The oral administration of the sample was done by oral gavage technique. The animals were handled with proper care and rehabilitated after the completion of the study.

Chemicals

Carrageenan and indomethacin for anti-inflammatory studies, Brewer's yeast for antipyretic studies, and Muller–Hinton agar medium and Rose Bengal chloramphenicol agar medium for antimicrobial studies were procured from Himedia Laboratories, Mumbai, Maharashtra, India. Other solvents and drugs used were of analytical grade and obtained commercially.

Antimicrobial assay

Antibacterial and antifungal activity studies were carried out by the agar diffusion method.[5] Petriplates containing 20 ml Muller–Hinton medium were seeded with 4 h culture of bacterial strains. Wells were cut, and 20 µl each of drug extract and the standard drug ciprofloxacin was added. The plates were then incubated at 37°C for 24 h. The antibacterial activity was assayed by measuring the inhibition zone formed around the well. Similarly, Petriplates containing 20 ml Rose Bengal chloramphenicol medium were seeded with a 12 h culture of fungal strains. Wells were cut, and 20 µl each of drug extracts and the standard drug fluconazole were added. The plates were then kept in room temperature for about 3–5 days. The antifungal activity was assayed by measuring the inhibition zone formed around the well.

Anti-pyretic activity

Healthy Swiss albino mice, *M. musculus* weighing 20 ± 5 g that have not been used for any previous experiments, were used for the study. Twenty-four Swiss albino mice were randomly selected and divided into six groups of four animals in each. The method of yeast-induced pyrexia

was applied in the study.[6] Before the induction of pyrexia, initial rectal temperature was recorded by using a digital thermometer. Group I served as normal control, which receives saline (5 mL/kg). Group II served as positive control, which receives paracetamol (150 mg/kg). Groups III and IV assigned for drugs has received varying doses (200 mg/kg and 400 mg/kg) of ethanol extract of NVK through oral route. Groups V and VI assigned for drugs has received varying doses (200 mg/kg and 400 mg/kg) of NVK capsules through oral route. Groups V and VI assigned for drugs received with varying doses (200 mg/kg and 400 mg/kg) of NVK capsules through oral route. The mice except the normal control were subcutaneously injected with 10 mL/kg of 20% aqueous suspension of yeast *Saccharomyces cerevisiae* and temperature was recorded at 1 h interval.

Anti-inflammatory activity-carrageenan-induced rat paw edema

Wistar rats of either sex weighing 190 ± 10 g used in this study were divided into six groups of four in each. 0.1 ml of 1% suspension of carrageenan in normal saline was injected in the right hind paws of the rats. Acute inflammation developed within 1 h after the injection. Group I served as control and received saline (5 ml/kg), Group II assigned as positive control and received Indomethacin (10 mg/kg), Groups III and IV assigned for treatment received the NVK in varying doses of 200 and 400 mg/kg, Groups V and VI received NVK capsules in varying doses of 200 and 400 mg/kg orally. The paw volume was measured using a Vernier caliper before (0 h) and at intervals of 1, 3, and 5 h after carrageenan injection. Then, the percentage of inhibition of edema was calculated for each group concerning the control group as follows,

Inhibition of paw edema (%) = (VC – VT/VC) ×100.

VC and VT represent the change in paw volume of control and treated animals, respectively.

Analgesic activity – acetic acid-induced writhing test

The Wistar rats of either sex weighing 190 ± 10 g were grouped similarly as seen in anti-inflammatory study. The method of Koster as modified by Danbisya and Lee was followed in this study.[7,8] The rats were subjected to fasting for 12 h before the experiment. Group I served as negative control (saline), Group II as positive control (acetylsalicylic acid, 100 mg/kg), Groups III and IV received NVK (200 and 400 mg/kg), and Groups V and VI received NVK capsule formulation (200 and 400 mg/kg) orally. After an hour, intraperitoneal injection of acetic acid (10 ml/kg, 0.6%) induced the abdominal constrictions. The number of writhing movements was counted for an hour. The number of abdominal constrictions in control groups and animals pretreated with test drugs was recorded.

The percentage against abdominal writhing was used to assess the degree of analgesia and was calculated using the following formula:

Inhibition (%) of writhing = (WC – WT/WC) × 100.

WC and WT represent the change in paw weight of control and treated animals, respectively.

Statistical analysis

All the data expressed as mean ± standard error of mean were evaluated by two-way analysis of variance followed by Tukey–Kramer as *post hoc* test, and *F* values of $P < 0.05$ were considered statistically significant.

Table 1: Antimicrobial assay

Microbial isolates	Zone of inhibition in diameter (mm)		
	Control	NVK-extract	NVK capsules
Escherichia coli	20±0.4	14±0.7	15±1.41
Pseudomonas aeruginosa	22±0.4	12±1.87	13±2.12
Klebsiella pneumonia	20±1.08	11±0.81	14±1.73
Proteus vulgaris	20±1.08	14±1.87	19±1.87
Staphylococcus aureus	23±0.7	16±0.7	17±1.41
Streptococcus epidermis	21±0.7	9±1.87	11±0.7
Shigella flexneri	22±1.08	15±0.7	16±2.44
Aspergillus flavus	20±0.4	16±1.08	18±1.41
Aspergillus niger	22±1.08	15±2.44	15±0.7

Values are expressed as mean±SEM of three replicates. NVK: *Nilavembu Kudineer*; SEM: Standard error of mean

RESULTS AND DISCUSSION

The antimicrobial assay in this study revealed that the zone of inhibition in NVK capsule-treated well is more than that treated with NVK ethanolic extract. The inhibitory zone of *S. epidermis, S. aureus, S. flexneri, P. aeruginosa, P. vulgaris, K. pneumonia, E. coli, A. flavus,* and *A. niger* is depicted in Figure 1. Table 1 shows the diameter of inhibitory zone (mm) in the control and study groups [Figures 1-9].

This study showed the better antimicrobial effect of NVK capsules than the NVK extract by their inhibitory action. The active components in the NVK capsule may be responsible for this effective antimicrobial activity. The zone of inhibition of micro-organisms ranges from 9 mm to 20 mm, which denotes a very better effect in comparison with control, which is from 20 mm to 25 mm. Moreover, NVK could be well prone to *E. coli* and *Shigella flexneri* infections because of its high inhibitory effect among all other microbes.

In previous studies, the *Nilavembu–A. paniculata* extract has been said to have a remarkable anti-diarrheal effect, which is caused by *E. coli* infections.[9] Similar studies reported that *Staphylococcus* is more susceptible to *Nilavembu – A. paniculata.*[10] The presence of the peptidoglycan layer in Gram-positive bacteria makes them more sensitive to plant extracts, which is more permeable to the extracts.[11,12] Based on the zone of inhibition, it was reported that the NVK capsule showed a greater antifungal effect against *A. flavus* compared to the NVK ethanolic extract.

It was observed that mice showed a marked rise in rectal temperature, 18 h after the Brewer's yeast injection. Administration of NVK at 200 mg/kg statistically significantly ($P < 0.01$) reduced pyrexia at 1–5 h compared to the control group animals. NVK at 400 mg/kg has only a moderate significance to that of the control group. Simultaneously, NVK capsule at 200 mg/kg better reduced the temperature and exhibited a statistically significant change ($P < 0.05$ to $P < 0.001$) in hyperthermia as almost equal to the control. Similarly at a dose of 400 mg/kg, the capsule was noted to have a significant reduction not lesser than NVK. This explains that the capsule exemplifies to produce a great significance than NVK [Table 2]. The results are illustrated in Graph 1.

Almost all the antipyretic drugs inhibit the enzymatic activity of cyclooxygenase (COX) and lower the prostaglandin PGE2 levels within the hypothalamic region.[13] Induction of pyrexia by yeast is known as pathogenic fever, which increases the synthesis of prostaglandins PGE2.[6] PGE2 is a potent hyperthermic agent and has been assigned an intermediary function in the response of thermoregulatory neurons to pyrogens.[6] The NVK capsule reduces the temperature better because of the presence of active molecules, which can enhance the production of natural antipyretic substances such as vasopressin and arginine by the body

and inhibit the prostaglandin synthesis. Flavonoid plays an important role in targeting prostaglandins which are involved in pyrexia.[14] The boiled and concentrated extract of NVK condensed into the capsule may have nanoparticles and large quantity of phytochemicals. The presence of large amount flavonoids and active nanoparticles in the capsule than the ethanol extract may contribute to its high antipyretic effect.

The NVK ethanol extract at both the doses has shown significance only at 1 h and 3 h in the study of anti-inflammatory activity ($P < 0.01$). Meanwhile, the NVK capsule at dose 200 mg/kg has exhibited a high statistical significance ($P < 0.01$) from 1 to 5 h in a time-dependent manner as compared to the control. In addition, similarly, the capsule at dose 400 mg/kg showed a better statistical significance at 5 h ($P < 0.05$) [Table 3]. Hence the capsule reduced the inflammation effectively than the NVK. The results are demonstrated in Graph 2.

Carrageenan-induced hind paw edema is the common experimental model of acute inflammation. Carrageenan, an agent to produce inflammation, plays a good role in testing anti-inflammatory drugs.[15] Carrageenan-induced edema is a biphasic event; in the early phase, there occurs the exhibiting of histamine, serotonin, and similar substances. In the later phase, the triggering of kinin-like substances and the release of prostaglandins, proteases, and lysosome take place.[16,17] The ingredients in NVK capsule have a different composition of phytochemicals, which contribute to the anti-inflammatory property and act on potential molecular channel by strongly reducing the production of the pro-inflammatory cytokines of interleukin (IL)-6 and IL-1 and the actively expressing COX-2 and simultaneously increasing the amount of anti-inflammatory cytokine IL-4 in the carrageenan-injected rat paw tissues.[16]

In the study of analgesic activity, the writhing effects were reduced well by NVK capsule than the extracts. The capsule caused a dose-dependent effect. Based on the doses tested, the capsule at 400 mg/kg showed a statistically significant effect ($P < 0.01$), reduced the painful response produced by acetic acid, and marked writhing compared to the control group. Similarly, the NVK at 200 mg/kg and capsule at 200 mg/kg showed significant analgesic effect, but the effect is not so as that of the capsule at 400 mg/kg; The results are showed in Graph 3 and [Table 4].

Acetic acid-induced writhing model constitutes pain sensation by activating a localized inflammatory effect. This causes the liberation of free arachidonic acid from phospholipids.[18] The effect is assumed to be mediated by peritoneal mast cells, acid-sensing ion channels, and the prostaglandin pathway.[18] The phytochemicals in drug particularly lowered the number of writhing, which is associated with exhibiting endogenous substances including serotonin, histamine, prostaglandin, and bradykinin. The results obtained thus suggest that the analgesic property of this drug is probably linked with its anti-inflammatory effect.

The present investigation proved that ethanolic extract and capsule form of NVK have significant antimicrobial, analgesic, antipyretic, and anti-inflammatory effects. The effectiveness of the capsule is more significant than NVK; the active constituents in capsules may have promoted its potential. The results of the antimicrobial study highlighted the potential of NVK capsule; in future, it may be exploited as a powerful antimicrobial agent against both Gram-positive and Gram-negative bacteria for treating nosocomial infections.

CONCLUSION

The study concludes that the portable and palatable form of NVK capsule of increased shelf-life is more beneficial while comparing with the extract of NVK. The researchers may further be undertaken to develop potent formulations consisting of NVK and its isolated molecules by making

Table 2: Antipyretic effect of *Nilavembu Kudineer* and capsule form of *Nilavembu Kudineer*

Treatment	Dose	Rectal temperature COX at various time intervals						
		−18 h	0 h	1 h	2 h	3 h	4 h	5 h
Control	5 ml/kg	37.41±0.04	38.48±0.04	38.76±0.06	38.61±0.07	38.71±0.04	38.71±0.04	38.71±0.04
Paracetamol	150 mg/kg	37.13±0.05	38.66±0.06	37.66±0.06*	37.46±0.10*	37.30±0.04*	37.25±0.02*	37.21±0.06*
NVK	200 mg/kg	37.33±0.13	38.56±0.12	37.68±0.10***	37.58±0.14	37.51±0.03	37.43±0.09	37.29±0.05*
	400 mg/kg	37.16±0.07	38.51±0.14	37.71±0.22**	37.63±0.15	37.46±0.07	37.37±0.11	37.33±0.14
NVK cap	200 mg/kg	37.22±0.03	38.36±0.03	37.60±0.02**	37.53±0.11*	37.43±0.07	37.32±0.08*	37.21±0.11***
	400 mg/kg	37.29±0.04	38.46±0.10	37.57±0.11	37.54±0.13*	37.41±0.04	37.35±0.09	37.28±0.06**
SE		0.047	0.058	0.182	0.178	0.227	0.227	0.132
F-test					2.88			

Each data expressed in mean±SEM; number of animals used (n=6). *P<0.05, **P<0.01, ***P<0.001 compared with positive control, considered as significant, more significant, and highly significant, respectively (two-way analysis of variance followed by Tukey-Kramer tests). SE: Standard error; SEM: Standard error of mean; NVK: *Nilavembu Kudineer*

Table 3: Anti-inflammatory effect of *Nilavembu Kudineer* and *Nilavembu Kudineer* capsule

Treatment groups	Rat paw edema volume at different time interval (mL)			
	0 h	1 h	3 h	5 h
Control-saline (5 mL/kg)	1.438±0.03	1.706±0.06	1.872±0.05	2.011±0.02
Indomethacin (10 mg/g)	1.429±0.01	1.524±0.03*	1.626±0.03**	1.665±0.07
NVK (200 mg/kg)	1.423±0.03	1.430±0.03**	1.562±0.07	1.775±0.05
NVK (400 mg/kg)	1.437±0.03	1.625±0.03	1.642±0.06**	1.785±0.05
NVK capsules (200 mg/kg)	1.423±0.03	1.417±0.04**	1.485±0.06**	1.620±0.05**
NVK capsules (400 mg/kg)	1.416±0.04	1.630±0.05	1.567±0.04	1.745±0.05*
SE	0.748	1.378	0.173	0.323

Each data expressed in mean±SEM; number of animals used (n=6). *P<0.05, **P<0.01 compared with positive control, considered as significant and more significant, respectively (two-way analysis of variance followed by Tukey-Kramer tests). SE: Standard error; SEM: Standard error of mean; NVK: *Nilavembu Kudineer*

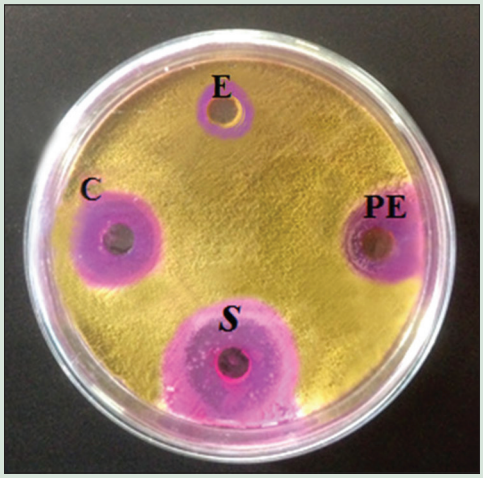


Figure 1: *Aspergillus flavus*

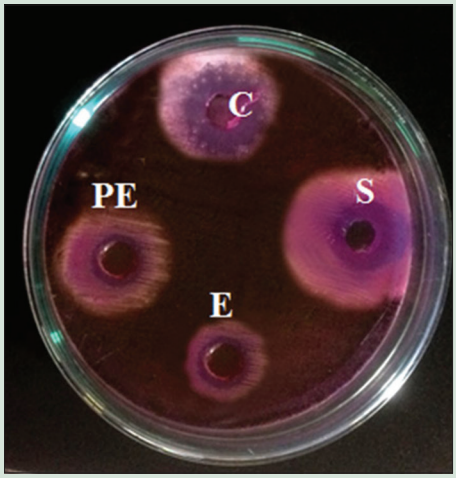


Figure 2: *Aspergillus niger*

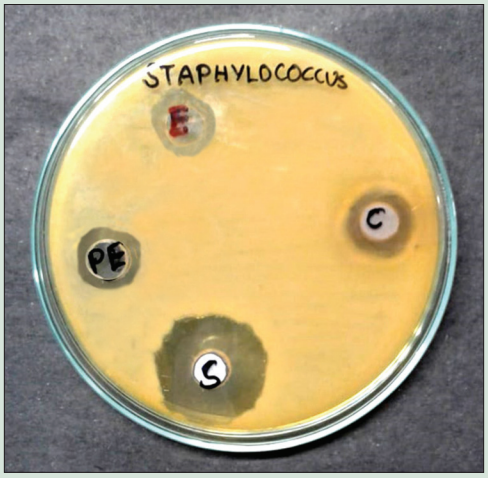


Figure 3: *Staphylococcus aureus*

Figure 4: *Proteus vulgaris*

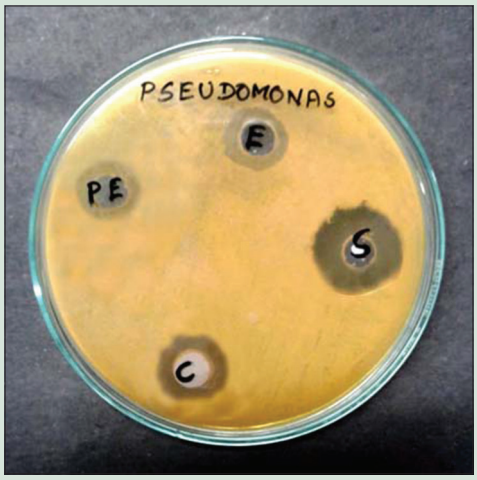


Figure 5: *Pseudomonous*

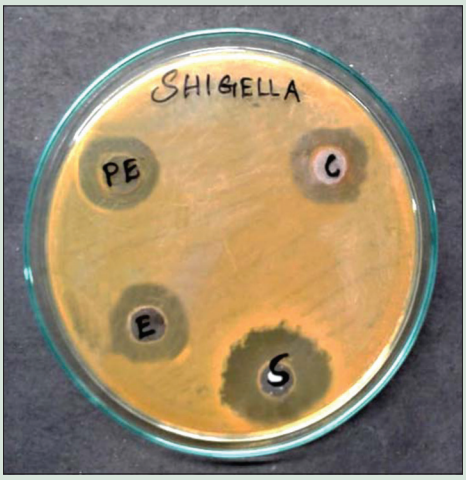


Figure 6: *Shigella*

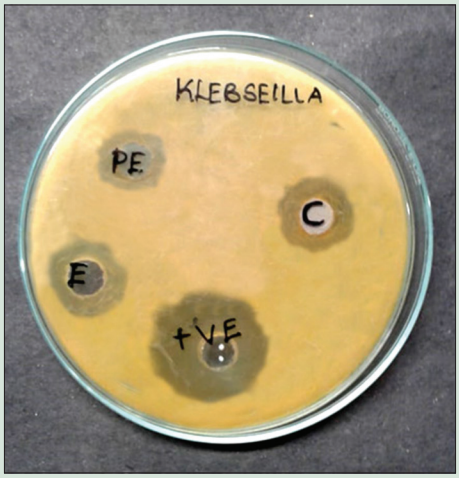


Figure 7: *Klebsiella pneumonia*

Figure 8: *Escherichia coli*

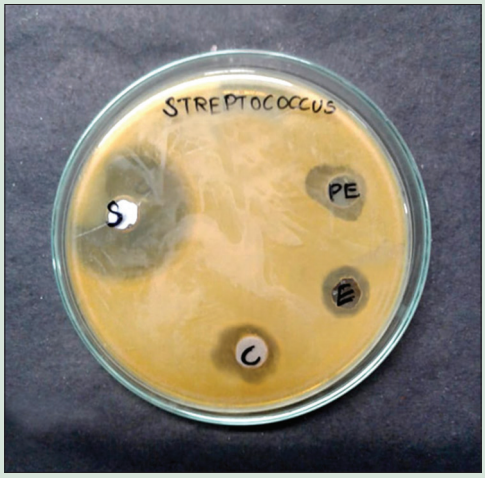
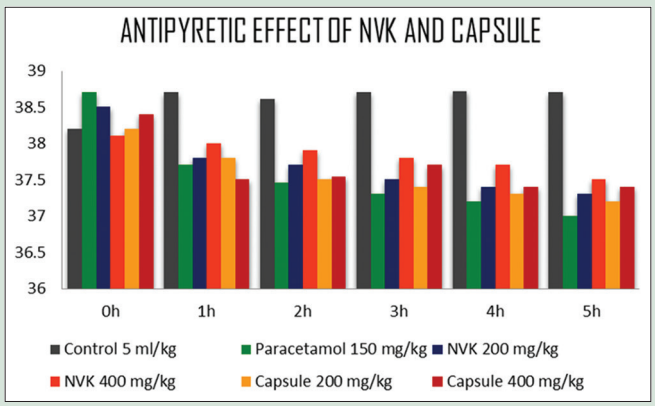


Figure 9: *Streptococcus*

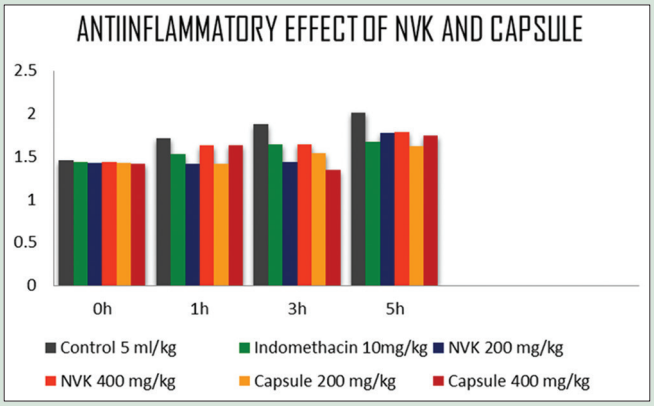


Graph 1: Graphical representation of anti-pyretic activity

Table 4: Analgesic effect of extract and capsule form of *Nilavembu Kudineer*

Treatment groups	Number of writhes in 1 h (mean±SEM)
Control-saline (0.1 mL/kg)	90.7±4.4
Acetyl salicylic acid (200 mg/kg)	44.50±2.30*
NVK (ethanol extract) 400 mg/kg	54±2.00*
NVK (ethanol extract) 200 mg/kg	48.16±1.59
NVK capsules 400 mg/kg	45±1.5*
NVK capsules 200 mg/kg	43±1.5
SE	0.998
F-test	*

All data were expressed in mean±SEM (n=6). *Significant P<0.01 compared with the control group. SE: Standard error; SEM: Standard error of mean; NVK: *Nilavembu Kudineer*



Graph 2: Graphical representation of anti-inflammatory activity

use of herbal drug delivery systems. Analysis on all the extracts and other pure phytochemicals isolated from this formulation is also important to ensure its safety and eligibility as a source of modern drug. The finding of the present investigation has also provided the scientific support to the classical Siddha formulation.

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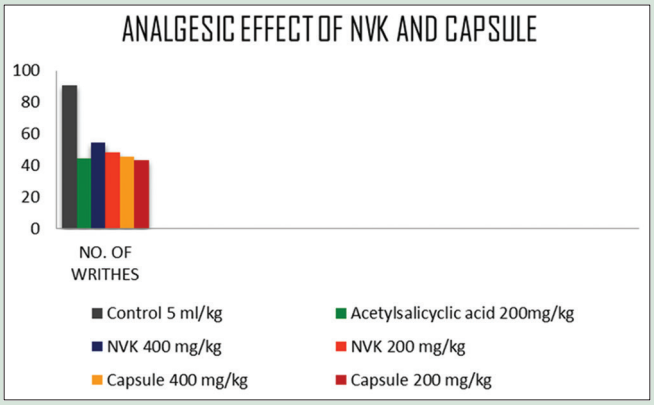
Nil.

Conflicts of interest

There are no conflicts of interest.



Graph 3: Graphical representation of analgesic activity

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