Acute and Chronic Anti-Inflammatory activity of Siddha Poly herbal Drug *Avuri Karpam*

Giftillda Selva Elsee T[1*], Manikgantan E M[2], Bharath Kumar[2] G

[1]Research Associate, National Institute of Siddha, Chennai.

[2]Department of Siddha, The Tamil Nadu Dr. M. G. R. Medical University, Chennai.

Abstract

Objectives:

The aim of the present study was carried out to document the acute and chronic anti inflammatory activity of *Avuri Karpam* (AK) in wistar albino rats.

Methods:

Acute anti inflammatory activity study was done using carrageenan induced paw edema method. In this study animals were divided into 5 groups with 6 animals in each group. Group-I (control) received 3% gum acacia 10 ml/kg p.o. Group-II (Carageenan) received 0.1ml of 1% w/v suspension of carrageenan S.C Group-III (standard) received Indomethacin 40 mg/kg p.o. Group-IV received AK 400mg/kg p.o. Group-V received AK 800mg/kg p.o. Chronic anti inflammatory activity were documented by using cotton pellet granuloma method. Totally, four experimental groups and six rats were employed for each experimental group. Group I is control. Group II received Indomethacin 20 mg/kg. Group III received AK 400mg/kg. Group IV received AK 800mg/kg.

Results:

The results of acute anti inflammatory activity study showed percentage Inhibition at 5 hours of standard drug treated group was 59%. AK 400mg/kg treated group had shown 42% of inhibition. Group V AK 800 mg/kg group showed 54% inhibition which was very close to the standard group inhibition. Chronic anti inflammatory activity result indicated that AK at the dose levels of 400 mg/kg and 800 mg/kg produced a significant decrease the weight of granuloma 46.2±5.66, 44.4± 2.22 respectively when compared with control group and percentage of protection follows in group II 35%, group III 15%, group IV 29.3%.

Conclusion:

Since it was concluded that Siddha drug has potent of acute and chronic anti inflammatory activity.

Keywords: Chronic inflammation, Siddha drug, Herbal medicine, Karpam, Arthritis.

*** Address of the Correspondence**

Dr. T. Giftillda Selva Elsee, Research Associate, National Institute of Siddha, Chennai -47.

Email Address: giftillda@gmail.com

INTRODUCTION:

Siddha system is a treasure house of secret science, embodying the results of the ordent pursuit thereof by the ancient Siddhars. This civilization dates back to 12,000 years B.C. The findings of historians and the Tamil literary works such as "*Tholkapiam,"* "*Thiruvasagam*", etc., reveal that there were three Tamil Academies for the growth of 64 Arts of Tamilians. Before 2000 years the traditional medical system of the Tamil was known as *Marunthu* (Medicine). [1]

Siddha medicine has been used traditionally to treat various diseases. Initially it is used in Tamil Nadu and now it is being used in many countries worldwide. Its protect everyone like a mother and it gives rejuvenation to the body. Holistic way approach is the best part of Siddha treatment. All treatment procedures help to normalize the altered life factors. Purgation normalizes *vatham*, emesis normalizes *pittham* and instillation normalizes *kabam*.

Karpam is one of the types of internal Siddha medicine. *Karpam* (rejuvenating/ elixir drugs) is a process in which leaves, herbs, roots, salts and minerals are consumed in a specific dose for a given period, along with the dietary regimen prescribed for it. They could be prepared daily or already prepared medicines could be used. [2] *Avuri Karpam* (AK) is a poly herbal formulation mentioned in Classical Siddha literature *Pathartha Guna Vilakkam* and it contains *Avuri ilai (Indigofera tincotria), Kaiyanthagarai (Eclipta alba), Kuppaimeni (Acalypha indica), Kottaikkaranthai (Spaeranthus indicus), Vallarai (Centella asiatica), Pottrilaikaiyanthagarai (Wedelia chinensis), Seruppadai (Coldenia procumbens)* indicated for treating *Keelvatham* (Arthritis), *Udhirakkattu, Sarpavisham.* [3]

The word "arthritis" means "joint inflammation." However, inflammation may also affect the tendons and ligaments surrounding the joint. The symptoms can develop gradually or suddenly and may impair a person's ability to perform everyday tasks. Inflammatory arthritis is characterized by damaging inflammation that does not occur as a normal reaction to injury or infection. This type of inflammation is unhelpful and instead causes damage to the affected joints, resulting in pain, stiffness, and swelling. [4] Inflammation is a reaction of living tissues towards injury, and it comprises systemic and local responses**. [5]**

Scientific validation of safety and efficacy of the each and every drug before going to administer in humans are essential in the current era. Standardization of drugs means confirmation of its identity and resoluteness of its standard and purity. Lack of quality control can affect the potency and well- being of drugs that may lead to health problems in the consumers. [6] *Avuri Karpam* has not been scientifically validated for arthritis. This present study was carried out to validate the acute and chronic anti-inflammatory in animal model.

MATERIALS AND METHODS

Drug Selection

The selected research drug *Avuri Karpam* is a siddha poly herbal formulation which has mentioned in siddha classical literature *"Pathartha Guna Vilakkam"* by *Kannu samy pillai* page no. 43.

Ingredients

1. *Avuri ilai (Indigofera tinctoria)* -50 g
2. *Kaiyanthagarai (Eclipta alba)* -50 g
3. *Kuppaimeni (Acalypha indica)* -50 g
4. *Kottaikkaranthai (Spaeranthus indicus)* -50 g
5. *Vallarai (Centella asiatica)* -50 g
6. *Pottrilaikaiyanthagarai (Wedelia chinensis)* -50 g
7. *Seruppadai (Coldenia procumbens)* -50 g

Collection of the Plant materials

Avuri ilai and *Seruppadai* were purchased from Rajendran Herbals, Thuckalay. *Kaiyanthagarai, Kuppaimeni, Vallarai and Pottrilai kaiyanthagarai* were collected from my native place (Tiruppattur) Herbal garden. *Kottaikkaranthai* was bought from the Ramasamy Mudhaliyar Store, Parry's corner, Chennai.

Identification and Authentication of the drug

All the plant materials were identified and authenticated by the experts of Siddha Central Research Institute (Central Council for Research in Siddha, Chennai, Ministry of AYUSH, Government of India), Govt. Anna Hospital Campus, Arumbakkam, Chennai -106

Purification of the drugs

Purification process was done as per classical Siddha literature. [7]

Method of Preparation

All the above purified ingredients were powdered separately and it was sieved by a cotton cloth.

- Then these powders were mixed together and it had gone for steaming process(*Pittaviyal murai*) for final purification.
- After this, the powder was dried and sieved again and stored in a clean air tightglass container for study purpose.
- It was labeled as *Avuri Karpam* (AK).

Administration of the Drug

Nature of the medicine : *Karpam*
Route of Administration : Oral
Dose : 800-1000 mg (twice a day)
Vehicle : Honey

Studies were conducted after obtaining prior approval No. 07/321/PO/Re/S/01/CPCSEA for animal studies from CPCSEA, Government of India through the Institutional Animal Ethics Committee (IAEC) of C.L. Baid Metha College of Pharmacy, Chennai – 97, Tamil Nadu, India.

Acute Anti-inflammatory activity of *Avuri Karpam* (AK) - Carrageenan induced rat paw oedema [8, 9]

For the experiment, the animals were divided into 5 groups with 6 animals in each group. Group-I (control) received 3% gum acacia 10 ml/kg p.o. Group-II (Carageenan) received 0.1ml of 1% w/v suspension of carrageenan S.C Group-III (standard) received Indomethacin 40 mg/kgp.o. Group-IV (Test-1) received AK 400mg/kg p.o. Group-V (Test-2) received AK 800mg/kg p.o. Grouping of animals were illustrated in Table – 1. All the drugs were administered orally and the volume of medicaments kept constant at10 ml/kg body weight of the animals it was administered orally to rats 1 hour before subcutaneous injection of carrageenan. After 1 hour 0.1ml of 1% w/v suspension of carrageenan was injected into sub-plantar region of the left hind paw to all the groups. The paw volume was measured at 1, 2, 3, 4, and 5 hour using Plethysmometer (Model 7150 UGO Basile, Italy) Oedema was expressed as the mean increase in paw volume relative to control animals.

Chronic anti-inflammatory activity of *Avuri Karpam* (AK) - Cotton pellet induced granuloma method [10]

Chronic anti-inflammatory activity of *Avuri Karpam* (AK) was determined using cotton pellet-induced granuloma model as per the standard method. Briefly, sterile autoclaved two cotton pellets weighing 7 mg implanted to anesthetized Wister albino rat in the dorsal region of each axilla. Totally, four experimental groups and six rats were employed for each experimental group. Group I is control. Group II received Indomethacin 20 mg/kg. Group III received *Avuri Karpam* (AK) 400mg/kg. Group IV received *Avuri Karpam* (AK) 800mg/kg. Grouping of animals were illustrated in table – 2. Further, 0.1% CMC was utilized as vehicle solution administered through per oral route. All experimental animals were maintained in standard conditions up to 7 days after cotton pellet implantation. On the 8th day, all animals were sacrificed using excess thiopental sodium to collect granulomatous tissues formed around the cotton pellet. The wet and dry weight ofgranulomatous tissues was recorded for each experimental animal group. Result values are statistically analyzed by Dunnett's "t" test using GraphPad 9 software and the values were considered significant at $P < 0.05$.

RESULTS

Acute anti-inflammatory activity of *Avuri Karpam* (AK)

Acute anti-inflammatory potential of siddha poly herbal formulation Avuri Karpam (AK) and the standard drug Indomethacin on the carrageenan induced hind paw edema was tabulated in table - 45. Volume of paw reduced in carrageenan induced hind paw edema in wistar albino rats illustrated in table – 46 and fig. 58. Percentage inhibitions at 5th hour were shown in fig. 59.

Injection of carrageenan induced a progressive edema reached maximum at 5th hour. Group I paw thickness showed 1.20 ± 0.14 and it remained constant at the end of 5 hours. Group II rats had showed increase in paw edema volume at each hour 1.91 ± 0.21, 2.37 ± 0.02, 2.47 ± 0.14, 2.58 ± 0.18, 2.63 ± 0.17 at 1, 2, 3, 4, 5 hours respectively.

The paw thickness of group III which received Indomethacin (40 mg/kg) is at initial stage 1.01± 0.06 where as it increased at 1 hr as 2.12 ± 0.26, and decreased to 1.52 ± 0.15, 1.45 ± 0.05, 1.32 ±0.18, 1.08 ± 0.16 at the end of 2, 3, 4, 5 hours respectively.

Group IV showed an increase up to 3rd hour and the thickness was found 1.66 ± 0.22. At the end of 5th hour it was decreased to 1.53 ± 0.24. Group V results revealed at the first hour the paw thickness was increased to 2.01 ± 0.22 and it was decreased at the end of 5th hour as 1.21 ± 0.12.

Group IV and V indicated a statistically significant decrease in paw volume ($p < 0.001$). Difference in paw volume between initial stage and 5th hour as 0.07, 0.19, 0.03 in respective groups III, IV and V.

% Inhibition at 5 hours of standard drug treated group was 59%. AK40mg/kg treated group had shown 42% of inhibition. Group V AK 800 mg/kg group showed 54% inhibition which was very close to the standard group inhibition.

Chronic anti – inflammatory activity of *Avuri Karpam* (AK)

The anti – inflammatory effect of the Siddha poly herbal formulation *Avuri Karpam* (AK) was assessed by using cotton pellet granuloma method in rats tabulated in table – 47 (Fig. 60). Effect of AK on % protection in wistar albino rats tabled in table – 48 (Fig. 61).

AK showed significant anti – inflammatory effect at the dose level of 400 and 800 mg/kg. Among the two doses, 800mg/kg showed maximum decreased of granuloma tissue formation. The result indicates that AK at the dose levels of 400 mg/kg and 800 mg/kg produced a significant decrease the weight of granuloma 46.2□5.66, 44.4□2.22 respectively when compared with control group and % of protection follows in group II 35%, group III 15%, group IV 29.3%.

Among the test groups, high dose of AK treated group exhibited the significant percentage protection 29.3 % which was nearly close to the protection of standard drug Indomethacin 35 %.

DISCUSSION AND CONCLUSION

The anti-inflammatory property of AK was studied by using carrageenan induced pawedema method. Carrageenan is a strong chemical used for the release of inflammatory and pro inflammatory mediators (prostaglandins, leukotrienes, histamine, bradykinin, TNF – α). Carageenan induced rat paw edema model is a suitable test for investigating anti – inflammatory drugs, which has frequently been used to assess the antiedematous effect of the drug. [11]

The course of inflammation is tri phasic; the 1st phase at one hour involves the release of serotonin and histamine from mast cells, second phase 3 hours is kinins and the third phase 5th hour is mediated by prostaglandins, the cyclooxygenase products and lipoxygenase products.[12,13] Prostaglandins are main responsible for acute inflammation. The cyclooxygenase and lipoxygenase pathways play a pivotal role in the inflammatory process; the inhibition of lipoxygenase inhibitors. [14]

The results of group V AK 800 mg/kg treated experimental rats revealed at the first hour the paw thickness was increased to 2.01 ± 0.22 and it was decreased at the end of 5th hour as 1.21 ± 0.12.

Phyto constituents present in AK might be block the prostaglandins production and inflammatory pathway. AK is rich in alkaloids and phenolic compounds. AK might have inhibited the cyclooxygenase which synthesises prostaglandins. The possible mechanism of action of alkaloids might suppress the antigen and mitogen – induced lymphocyte proliferation, natural killer cell cytotoxicity, histamine release by mast cells, and interleukin-1 secretion by human monocytes. [15,16]

The mechanism of action of AK might be associated with the inhibition of histamine, serotonin and prostaglandin synthesis. As shown in table the reduction of edema in percentage with AK high dose treated group was 54% which was nearly equal to standard drug inhibition (59%) and indicating an anti-inflammatory effect. The result of present study indicates the efficacy at the dose level of 400 mg/kg and 800 mg/kg of AK as an efficient therapeutic agent in acute inflammatory conditions.

AK showed significant chronic anti – inflammatory effect at the dose level of 400 and 800 mg/kg in testing of cotton pellet granuloma method. The response to a subcutaneously implanted cotton pellet in rats could be classified into three phases as follows: transudative phase, exudative phase and proliferative phase. [17]The cotton pellet granuloma method is widely used to assess the transudative and proliferative components of inflammation. [18] Implanted cotton pellet materials induced inflammatory responses and they released the inflammatory mediators which lead to tissue damage and granular formation. The weight of the wet cotton pellets correlates with transudative material and the weight of the dry pellet correlates with the amount of granulomatous tissue. [19] The chronic inflammation occurs by means of the development of proliferative cells. These can be either spread or in a granuloma form.

Anti-inflammatory drugs can reduce transudative weight probably via their ability to inhibit the permeability response of the blood vessels around the cotton pellet implantation. They can also effectively inhibit the granuloma formation probably due to interference with proliferative components of inflammatory process. NSAIDs such as aspirin elicit a slight inhibition whereas steroidal anti – inflammatory drugs have a strong inhibition on both transudative and proliferative phases of inflammation.[17] Cotton pellet granuloma tends to minimise the chronic inflammation caused by non- microbial and non-degradable pathogens. A non-immunological type of inflammation was observed and it was mediated by the activation of inflammatory mediators such as kinins. [20]

Chronic inflammation associated with development of proliferate cells. NSAIDS reduce the volume of granuloma, reduce infiltration, inhibits the growth of collagen fibres and decreases mucopolysaccharides. [21, 22]

In this study, decrease in the weight of granuloma indicated that the proliferative phase was effectively suppressed by AK. Most of the ingredients of AK have anti- inflammatory properties. The phyto constituents present in the ingredients may reduce inflammatory conditions.

AK has been observed to inhibit the weight of granuloma tissue in a dose dependent manner and the high dose 800 mg/kg exhibited the inhibition of inflammation which was very close to standard drug protection. That is high dose of AK treated group exhibited the significant 29.3% of protection whereas the percentage protection of standard drug Indomethacin showed 35 %. This study exhibits remarkable anti-inflammatory activity in chronic inflammatory conditions. However in future it is must to identify and isolate the active chemical constituents which are responsible for the chronic anti-inflammatory actions. In conclusion, the present study clearly highlights AK has potent acute and chronic anti-inflammatory effects in animal model. However clinical trial have been recommended for further documentation of therapeutic benefits.

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TABLES

Table – 1: Grouping of animals for Acute Anti-inflammatory activity of Avuri Karpam (AK)

Groups	No. of rats
Group- I Control (3% gum acacia 10 ml/kg.)	6
Group- II 0.1ml of 1% w/v suspension of carrageenan	6
Group-III Indomethacin 40 mg/kg p.o	6
Group- IV AK (400 mg/kg)	6
Group- V AK (800 mg/kg)	6

Table – 2: Grouping of animals for Chronic anti-inflammatory activity of *Avuri Karpam* (AK)

Groups	No. of rats
Group- I Control	6
Group-II Indomethacin 20 mg/kg p.o	6
Group- III AK (400 mg/kg)	6
Group- IV AK (800 mg/kg)	6

Table – 3 Acute anti-inflammatory activity of *Avuri Karpam* (AK) on carrageenan induced hind paw edema in wistar albino rats

	Initial paw volume	Change in paw edema mm at different time intervals					
	0 hr	1 hr	2 hrs	3 hrs	4 hrs	5 hrs	% Inhibition at 5 hrs
I Control	1.20 ±0.14	1.20±0.14	1.20±0.14	1.20±0.14	1.20±.14	1.20±0.14	-
II Carrageenan	1.21±0.17	1.91±0.21	2.37±0.02	2.47±0.14	2.58±0.18	2.63±0.17	-

III Indomethacin 40mg/kg	1.01±0.06	2.12±0.26	1.52±0.15***	1.45±0.05***	1.32±0.18***	1.08±0.16***	59%
IV AK 400 mg/kg	1.34 ±0.11	1.47 ±0.32	1.53±0.18**	1.66±0.02**	1.55±0.22**	1.53 ± 0.24***	42%
V AK 800 mg/kg	1.24±0.42	2.01±0.22	1.91±0.23***	1.67±0.44***	1.61±0.18***	1.21±0.12***	54%

n=6; Statistical analysis one way ANOVA followed by Dunnett t-test. All the groups were compared with group II *p<0.05, **p<0.01,***p<0.001. The paw volume up to the tribiotural articulation was measured at 0, 1, 2, 3, 4, 5 hrs.

Table – 4. Volume of paw reduced in carrageenan induced hind paw edema in wistar albino rats

Group	Initial paw volume	5 hr	Difference in paw volume
I	1.20 ± 0.14	1.20±0.14	0.00
II	1.21± 0.17	2.63 ± 0.17	1.42
III	1.01± 0.06	1.08 ± 0.16	0.07
IV	1.34 ± 0.13	1.53 ± 0.32	0.19
V	1.24 ±0.42	1.21 ± 0.12	0.03

Table – 5. Chronic anti-inflammatory activity of *Avuri Karpam* (AK) in wistar albino rats by using cotton pellet granuloma method

Groups	Wet weight (mg)	Dry weight (mg)	Transudative weight (mg)
I Control	217.4±7.54	57.2±4.08	160.2
II Indomethacin 20mg/kg	155.4±8.10	52.3±4.42	103.1
III AK 400mg/kg	182.8±6.22	46.2±5.66	136.6
IV AK 800mg/kg	157.7±8.10	44.4±2.22	113.3

Table – 6 Effect of *Avuri Karpam* (AK) on % protection) in wistar albino rats

Groups	% protection
I Control	–
II Indomethacin 20mg/kg	35%
III AK 400mg/kg	15%
IV AK 800mg/kg	29.3%

Figures

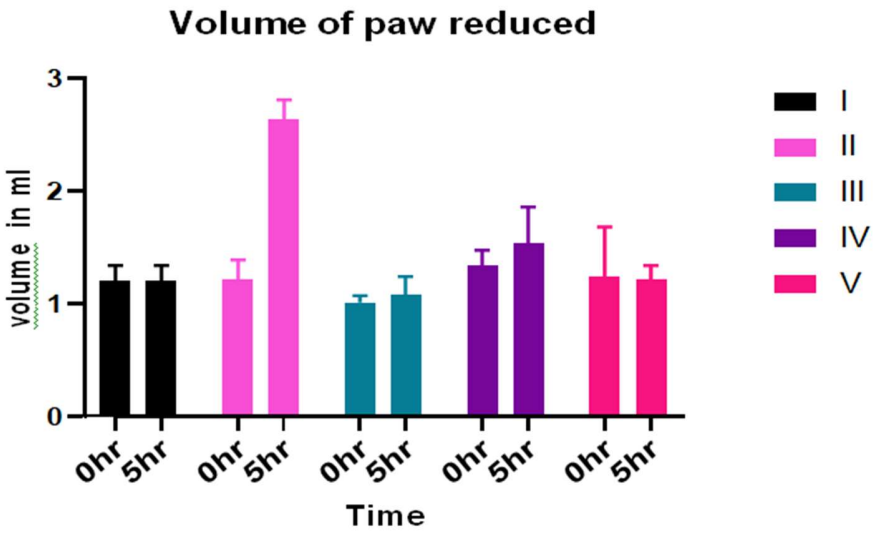

Fig. 1 Volume of paw reduced in carrageenan induced hind paw edema in wistar albino rats

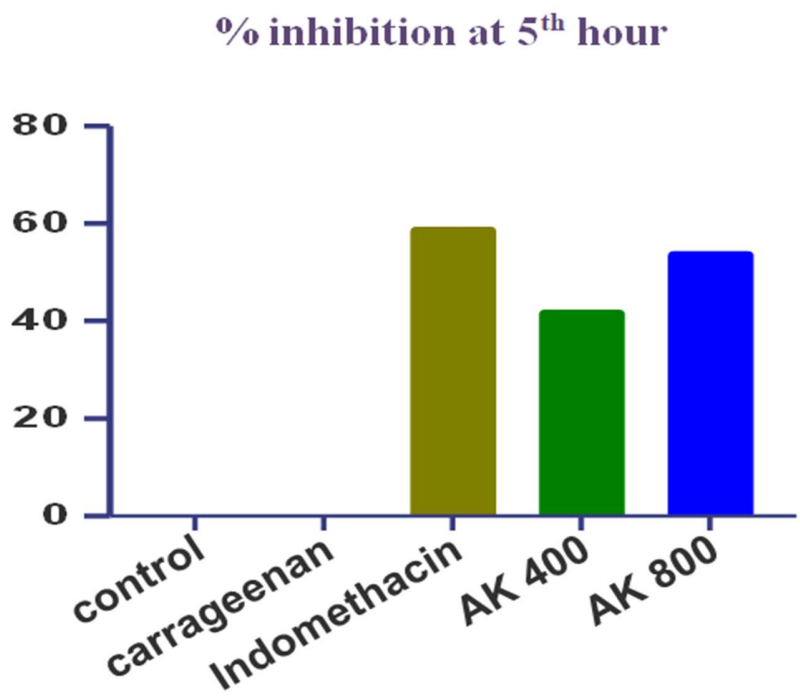

Fig. 2 Percentage inhibition at 5th hour on carrageenan induced hind paw edema in wistar albino rats

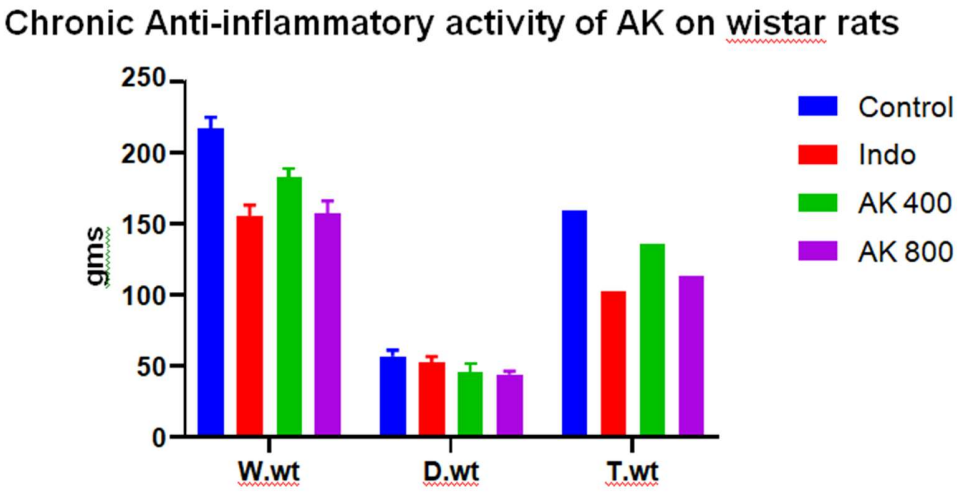

Fig. 3 Chronic anti-inflammatory effect of *Avuri Karpam* (AK)

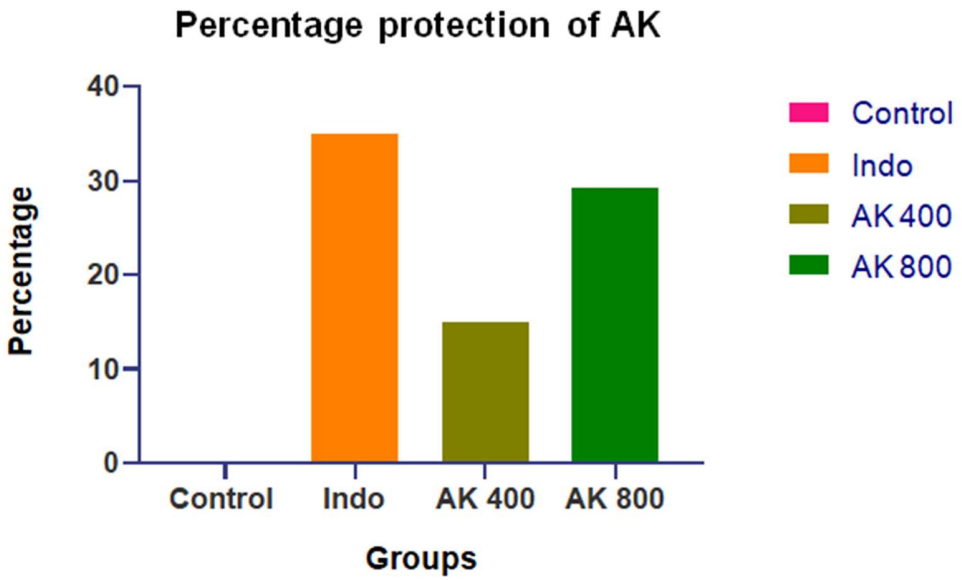

Fig. 4 Effect of *Avuri Karpam* (AK) on % protection