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Anti-Angiogenic Effect of *Caryota Urens* Fruits Extract on the Fin of Zebrafish

Authors
Vikram Shokin Bafna[1], Sughandha G. Chaudhari[2], Neethu R.[3], Rajendra Patil[4] Mukesh C.[5], Geeta B[6]
Dr. L.H. Hiranandani College of Pharmacy
Corresponding Author
Vikram Shokin Bafna
Dr. L.H. Hiranandani College of Pharmacy, Ulhasnagar-3, Mumbai, Maharashtra, India
Email: *vikramsbafna4689@gmail.com*

ABSTRACT

The aim of this study is to explore Caryota urens which may lead to discovery of new therapeutic agents which may inhibit process of angiogenesis and in future which may be utilized for treatment of various disease condition like cancer, cardiovascular diseases, arthritis, diabetic retinopathy etc. where angiogenesis play a major role in modulating disease processes. With this aim, the objectives of the study includes identification of active constituent which will be crucial in understanding the mechanism of action of the said activities and to evaluate the anti-angiogenic activity of the Caryota urens fruit extract in zebra fish fin regeneration model. Zebra fishes were maintained and fish regeneration assay was done on ethanolic extract of Caryota urens (EECU) and inhibition in regeneration of zebra fish fin was observed for over a period of thirty days with regular dosing on every alternate day. Fishes were divided into groups. The groups were treated with various concentrations of test and standard drug. Area of regenerated fin was measured frequently by observation under microscope and measurement by softwares. The dose response of test and standard were plotted and inhibition in regeneration was observed accordingly. The inhibition of regeneration of fin in zebra fish was observed in test drug which may be as a result of phytoconstituents present in Caryota urens. The present study represents that the zebra fish models can be applied for studying anti-angiogenic activity and Caryota urens have potential for further exploring for its application in inhibition of process of angiogenesis.

Keywords:- *Fruit extract, zebrafish, fin regeneration, angiogenesis.*

1. INTRODUCTION

Angiogenesis is the process of new vessels development from the pre existing blood vessels. This important mechanism is essential for organ growth during embryonic development and wound healing in adults. An adequate supply of nutrients and oxygen supply is necessary for homeostasis and normal growth of cells and tissues. This delivery of oxygen and nutrients and removal of metabolic waste and carbondioxide depends on blood vessels. This important mechanism have important role in many clinical conditions. The inhibition may of this process lead to various conditions like ischemic disease, neurodegeneration, hypertention, etc, while its excessive stimulation leads to diseases like arthritis, blindness, atherosclerosis, cancer, etc. although there are various synthetic drugs available which are useful in treatment of above mentioned disease but they also have side-effects[1]. On the other hand herbal drugs also have been used which are comparatively more safer then synthetic drugs. The phytoconstituents present in herbal drugs also forms part of physiological system. Hence this can be applied for exploration for inhibition of process of angiogenesis[1].

On the basis of presence of high antioxidant activity and chemical constituents which already have established anti-angiogenic activity we selected *Caryota urens* for present study[2]. The flowers and fruits have rich source of phytochemicals, antioxidant capacity. The presence of chemical constituents like epigallocatechin gallate, epicatechin gallate, catechin and gallic acid in caryota urens is the basis for selection of this plant for study[2][3].

Zebrafish is an excellent model as its circulatory system resembles that of mammals. This models are effective, robust, reproducible and cheaper then conventional models. Zebra fish have capacity to regenerate their fin, optical nerve, retina etc when amputed [4]. In this study we used ethanolic extract of fruits of *Caryota urens* on zebra fish regeneration model for study anti-angiogenic activity.

1.1 PROCEDURE

Preparation of extract

The ripe fruits of *Caryota urens* were collected and authenticated. The fruits were dried and powdered. The powder was extracted with ethanol by soxhlet extraction method for 48hrs. The extract was collected and concentrated with the help of rotator evaporator. The extract was dried and stored in air tight containers for further use. Water soluble extract was used for study of anti angiogenic activity[5].

2. MATERIALS AND METHODS

Fish husbandry and methods

Fishes were obtained from local suppliers. They were acclimatize for upto two weeks at 25ºC at appropriate light and dark light cycle. Fishes were fed three times a day with flake food[6][7].

Stock solutions of extract were prepared in distilled water and standart in indistilled water with DMSO as a co-solvent. The stocks were stored in cool in light resistant containers.

Toxicity test

Toxicity test was conducted to calculate the maximum tolerable dose for standard and test drugs using Up and Down method[8]. This studies were

helpful in determining the doses for study. Fish water was prepared with sodium thiosulphate and rock salt dissolved in tap water.

Test stock solution:- Test stock solution of dried ethanolic extract of *Caryota urens* was prepared as 1mg/ml concentration in distilled water in volumetric flask.

Standard stock solution:- Standard stock solution of Paclitaxel (PAC) was prepared as 1mg/ml concentration in distilled water with 1.5% DMSO as a co-solvent in volumetric flask.

One fish per dose was kept in 150ml of fish water in 250ml beaker with continues aeration and observed for lethality for 24hrs. Based on the mortality, dose was increased or decreased by the factor log0.5 or 3.2.

EXPERIMENT

Adult fish obtained from the local suppliers were kept in large tanks for acclimatization with continues supply of air. On the day of experiment, fish were taken out and they were placed in 150ml of fish water in 250ml beakers divided into 10 treatment groups: 6 fish in each group. They were anesthetized using 0.1% 2-phenoxyethanol[9]. Their fin was cut up to 50% by using sterilized straight razor blade and imaged under microscope[10]. Pre and post amputation images were collected before transferring the fishes in recovery beaker containing fish water. Test drug, and standard drug were given to different groups, control and vehicle control were maintained in similar conditions like test and standard. The fish water was changed on every alternate day and dose was renewed. This was done for 30 days till full regeneration of fin is obtained normally[11][12].

The images of amputed fin was collected on day 10, day 20, and day 30. The area of regenerated fin was calculated using imagej software. Percentage of regeneration was calculated and significance was obtained statistically.

Table:1 Treatment dose table

Treatment Group	**Dose of drug**
Control	Fish water
Vehical control	1.5% DMSO in fish water
Standard group 1(PAC)	5μg/150ml standard drug Paclitaxel
Standard group 2(PAC)	7.5μg/150ml standard drug Paclitaxel
Standard group 3(PAC)	10μg/150ml standard drug Paclitaxel
Test group 1(EECU)	5μg/150ml ethanolic extract of *Caryota urens*
Test group 2(EECU)	10μg/150ml ethanolic extract of *Caryota urens*
Test group 3(EECU)	20μg/150ml ethanolic extract of *Caryota urens*

3. RESULT AND DISCUSSION

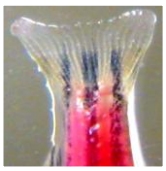
Toxicity

Table:2 Toxicity and dose determination

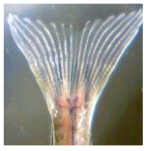
Group	**Lethal dose**
Standard	10μg/150ml
Test	25μg/150ml

Depending on above data test doses and standard doses were selected.

OBSERVATIONS

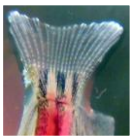

Control day 10

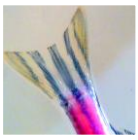

Control day 30


Vehicle control day 10

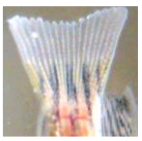

Vehicle control day 30

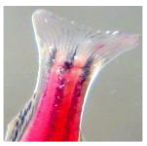
Figure:1 Observations for Control, and Vehicle control on day 10 and day 30.

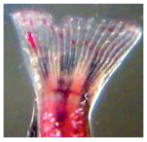

PAC 2.5µg/150ml day 10

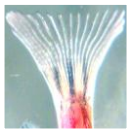

PAC 2.5µg/150ml day 30

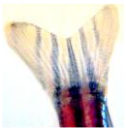

PAC 5µg/150ml day 10

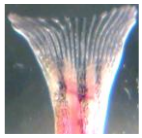

PAC 5µg/150ml day 30

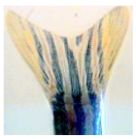

PAC 7.5µg/150ml day 10


PAC 7.5µg/150ml day 30


EECU 5µg/150ml day 10


EECU 5µg/150ml day 30


EECU 10µg/150ml day 10


EECU 10µg/150ml day 30


EECU 20µg/150ml day 10


EECU 20µg/150ml day 30

Figure:2 Observations for Standard dose (PAC), Test dose (EECU), Control, and Vehicle control on day 10 and day 30.

Statistical analysis

Table:3 Percent regeneration of caudal fin on Day 10, 20, 30 by PAC, EECU Control, and Vehicle control.

Days	Mean ± Sem			
	Control	Vehicle Contol	PAC 7.5μg/ 150ml	EECU 20μg/ 150ml
10	91.25± 2.314	90.47±1 .818	45.95±3.5 77***	54.58±5.1 76***
20	97.24± 0.9367	95.90±1 .583	48.85±3.4 81***	66.25±5.6 96***
30	98.79± 0.4037	98.21±0 .4858	59.19±4.7 99***	81.37±7.6 06*

All values are expressed as mean ± SEM for n=6 animals. P values *p< 0.05, when compared to control group using One-way ANOVA followed by Dunnett's test.

The regeneration was significantly inhibited by Test dose 20μg/150ml of EECU and Standard dose of 5µg/150ml and 7.5μg/150ml PAC on day 10, day 20 and day 30. While control and vehicle control fish regenerated their caudal fins in 30 days after amputation. Thus ethanolic extract of *Caryota urens* at dose of 20µg/150ml showed a significant inhibition of regeneration of fin as compared with control and vehicle control. While the regeneration was maximum with complete growth of fin in control and vehicle control groups.

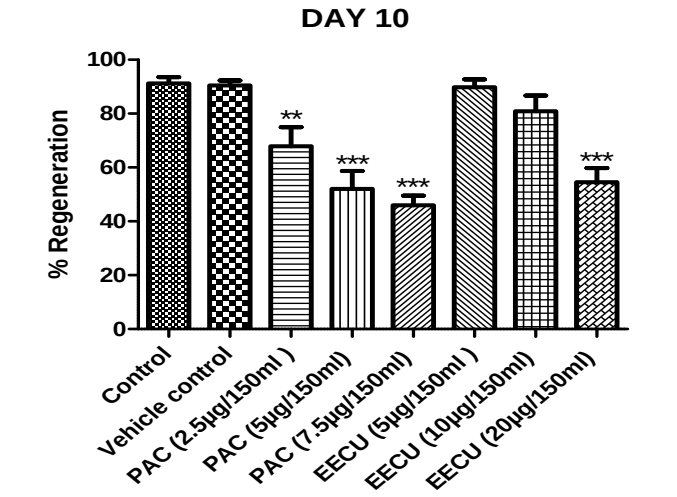


Figure:3 Graph for % Regeneration on Day 10 of zebrafish fin control vehicle control vs PAC vs EECU.

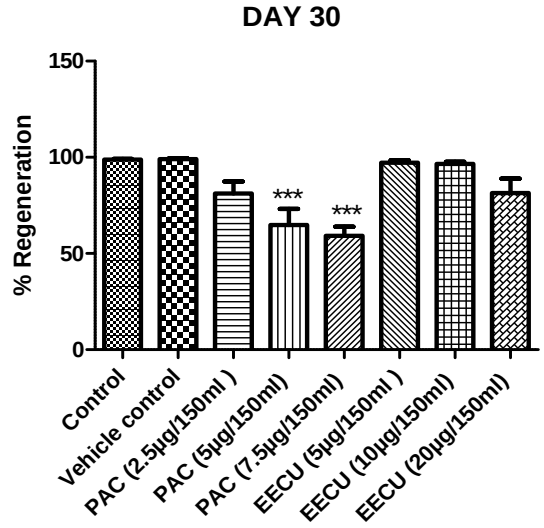


Figure:4 Graph for % Regeneration on Day 30 of zebrafish fin control vs vehicle control vs PAC vs EECU.

4. CONCLUSION

Thus in above study zebra fish was used as anti-angigenic model to study the effect of ethanolic extract of *Caryota urens* on inhibition of process of angiogenesis. Percent of regeneration was calculated applied for statistical analysis using one way ANOVA and imageJ software. The results obtained showed that the ethanolic extract of *Caryota urens* have significant anti-angiogenic activity when compared with control, vehical control and Standard for period of 30 days. Thus the chemical constituents from *Caryota urens* can further studied after isolation and separation of individual constituents. Thus this plant can be explored further for its potential anti-angiogenic activity for treatment of variety of ailments in which angiogenesis plays crucial role.

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