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EVALUATION OF STYPTIC ACTIVITY OF NAAGA SANGU PARPAM IN WISTAR RATS

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ABSTRACT

The aim of present study is to evaluate the styptic activity of Naaga sangu parpam. Animals were divided into three groups and each group contains six animals. First group received 1ml of Ghee, second group received 10 mg/kg of Adrenochrome, third group and fourth group received 12mg/kg and 24mg/kg of *Naaga Sangu Parpam* respectively. After 1 hour, the following parameters such as clotting time, bleeding time, prothrombin time, activated partial prothrombin time and fibrinogen time were screened. *Naaga Sangu Parpam* at both 12mg/kg and 24mg/kg exhibited significant (***P < 0.001) Styptic activity.

KEYWORDS: Naagasangu parpam, styptic, adrenochrome.

INTRODUCTION

Piles means a ball or mass, Haemorhoids means blood to ooze. It is abnormal sliding downwards of anal cushions due to straining or other causes.[1] Hemorrhoids are clusters of vascular tissues, smooth muscles, and connective tissues that lie along the anal canal in three columns—left lateral, right anterior, and right posterior positions. Because some do not contain muscular walls, these clusters may be considered sinusoids instead of arteries or veins.[2] The present treatment includes Hemorrhoidectomy, Doppler guided hemorrhoidal artery ligation, stapled hemorrhoidopexy, sclerotherapy, infrared coagulation, rubber band ligation.[3] Although hemorrhoids are recognized as a very common cause of rectal bleeding

and anal discomfort, the true epidemiology of this disease is unknown because patients have a tendency to use self-medication rather than to seek proper medical attention.[4] Arrest of bleeding is important in the treatment of bleeding piles. For a drug to arrest bleeding styptic property is important. In this purview, Styptic activity Naagasangu parpam was evaluated. of The drug *Naaga Sangu Parpam*, is a Siddha herbo-metallic preparation, mentioned in *Siddha* text *Kannusamy paramparai vaithiyam*, pg.no: 414, indicated for *Moolam* (Hemorrhoids), *Powthiram* (Fistula in ano), *Vellai* (Leucorrhoea) and *Vettai* (Venereal disease).[5]

MATERIALS AND METHODS

Collection of the raw drugs

Naagam and *Sangu* were procured from a well reputed country shop in Parrys, Chennai. *Uthamani* was freshly collected from Tambaram sanatorium. *Naagam and Sangu* were purified and the medicine was prepared in the *Gunapadam* laboratory of National Institute of Siddha.

Identification and Authentication of the drug

Sangu (Conchshell) was authenticated at Marine Biology Regional Centre, Zoological Survey of India, Chennai. Metal drug *Sangu* (Zinc) was authenticated at Department of Geology, University of Madras, Chennai. *Pergulaeria daemia* Linn. was Identified and authenticated by Botanist, National Institute of Siddha, Tambaram Sanatorium, Chennai.

Selection of animals

Healthy Wistar albino rats (200-250gm) of both sex were used for this study with the approval of the Institutional Animal Ethics Committee and obtained from the animal laboratory. IAEC approved no: NIS/IAEC-III/04/29092016.

The animals were kept in plastic cages and maintained at 24-28°C. All the rats were housed individually with free access to food, water and libitum. They were fed with standard diet and kept in well ventilated animal house and they were also maintained with alternative dark-light cycle of 12 hrs throughout the study. Rats were allowed an acclimatization period of 7 days before actual experiment. The rats were closely observed for any infection and if they show any signs of infection they were excluded from the study. The animal experiment was performed with accordance legislation on welfare.

EXPERIMENTAL DESIGN

Experimental procedure

The animals were divided into 4 groups of six animals in each group. The animals were kept overnight fasting before the experiment. First group received 1ml of Ghee, second group received 10 mg/kg of Adrenochrome, third group and fourth group received 12mg/kg and 24mg/kg of *Naaga Sangu Parpam* respectively. After 1 hour, the following parameters such as clotting time, bleeding time, prothrombin time, activated partial prothrombin time and fibrinogen time were screened.

Clotting time

The tail of the animal was warmed for 1 min in water at 40°C, dried and cut at the tip with a razor blade. A 25µl sample of blood was collected into a micro hematocrit glass capillary tube. The time of appearance of the first drop of the blood on the cut tail was noted. The blood was left to flow by gravity between the two marks of the tube, 45 mm apart, by 77 tilting the capillary tube alternately to +60° and -60° angles with respect to the horizontal plane until blood ceased to flow (reaction end point). The glass tube was then kept between the palms of both the hands for 30 seconds to maintain it at body temperature. After 30 seconds the tube was taken out and small portion of the capillary tube was broken at regular intervals of 10 seconds, until a thread of clotted blood appears between the two pieces of capillary glass tube. The time interval between the appearance of the drop of the blood and the thread of the blood clot was noted as the clotting time of rat expressed in minutes.

Bleeding time method (BT)

The tail of the rat was warmed for 1min in water at 40°C and then dried. A small cut was made in the middle of the tail with a scalpel. Bleeding time was calculated from the time of first blood appeared till bleeding was stopped. Spots were made with the bleeding tail on a blotting paper every 15 seconds, till the bleeding stopped and the bleeding time was calculated accordingly.

Animal blood collection

For the remaining parameters like prothrombin, activated partial prothrombin time and fibrinogen time, blood was collected from the lateral tail vein. The blood sample was immediately emptied into a plastic tube containing 0.11M sodium citrate at a ratio of 1:10 anticoagulant blood, gently mixed and centrifuged at 2500g at 4c for 10 min. The plasma was separated and maintained in ice bath throughout this process.

Prothrombin time (PT)

0.1 ml of plasma was mixed with 0.2 ml of PT reagent (calcium thromboplastin) and maintained at 37°C and observed until the formation of fibrin clot and the time was noted.

Activated Partial Thromboplastin time (APTT)

0.1ml of plasma was mixed with 0.1ml of APTT reagent (cephalin-karolin suspension) and was incubated at 37°C for 5 minutes and then 0.1ml of 0.025ml cacl2 solution was added and observed until the formation of fibrin clot visually detected and the time was noted.

Fibrinogen time

0.25ml of animal blood plasma was mixed with 0.05 ml of saline and was incubated at 37°C. After 30's, 0.1ml of streptokinase solution was added and waited for 30's, then 0.1ml of bovine thrombin was added. The Stopwatch was started. 30 or later, the time when the fibrinogen clot formed was noted.

Statistical analysis

N= 6, Values are expressed as mean ± SD, analysis was done by using One-Way ANOVA followed by Dunnett's Test. Test for significance is *P < 0.05, **P < 0.01, ***P < 0.001.

RESULTS AND DISCUSSION

Bleeding Time (BT)

In the vehicle treated control group the mean bleeding time is 4.89±0.35, while NSP at 12mg/kg it is 3.82±0.28*** and at 24mg/kg it is 3.07±0.82***. The result showed that *Naaga Sangu Parpam* at both 12mg/kg and 24mg/kg have more significant (***P < 0.001) effect, when compared to control group (Fig.1).

Clotting time (CT)

The mean clotting time in this vehicle treated control group is 4.55±0.34, while NSP at 12mg/kg it is 2.84±0.31*** and at 24mg/kg it is 2.32±0.11***. The result showed that *Naaga Sangu Parpam* at both 12mg/kg and 24mg/kg have more significant (***P < 0.001) effect, when compared to control group (Fig.1).

Prothrombin time (PT)

In the vehicle treated control group the mean prothrombin time is 11.97±0.47seconds while NSP at 12mg/kg it is 10.2±0.32***and at 24mg/kg it is 10.5±0.57***. The result showed that

Naaga Sangu Parpam at both 12mg/kg and 24mg/kg have more significant (***P < 0.001) effect, when compared to control group (Fig.1).

Activated Thromboplastin

The mean Activated Thromboplastin time in this vehicle treated control group is 29.88±0.32, while NSP at 12mg/kg it is 27.6±0.48***and at 24mg/kg it is 24.9±0.59 The result showed that *Naaga Sangu Parpam* at both 12mg/kg and 24mg/kg have more significant (***P < 0.001) effect, when compared to control group (Fig.2).

Fibrinogen time

The mean Fibrinogen time in this vehicle treated control group is 175.6±7.36, while NSP at 12mg/kg it is 202.17±19.14*** and at 24mg/kg it is 223.8±16.86***. The result showed that *Naaga Sangu Parpam* at both 12mg/kg and 24mg/kg have more significant (***P < 0.001) effect, when compared to control group (Fig.3).

Table 1: Styptic activity of *Naaga Sangu Parpam* by Tail cutting method.

S.No	Parameters	Control	Group I	Group II	Group III
1	Bleeding time (s)	4.89±0.35	2.96±0.26***	3.82±0.28***	3.07±0.82***
2	Clotting time (s)	4.55±0.34	2.84±0.08***	2.84±0.31***	2.32±0.11***
3	Prothrombin time(s)	11.97±0.47	8.55±0.26***	10.2±0.32***	10.5±0.57***
4	Activated Thromboplastin time (s)	29.88±0.32	22.51±0.73***	27.6±0.48***	24.9±0.59***
5	Fibrinogen time(s)	175.6±11.17	271±11.17***	202.17±19.14***	223.8±16.86***

N= 6, Values are expressed as mean ± SD, analysis was done by using One-Way ANOVA followed by Dunnett's Test. Test for significance is *P < 0.05, **P < 0.01, ***P < 0.001.

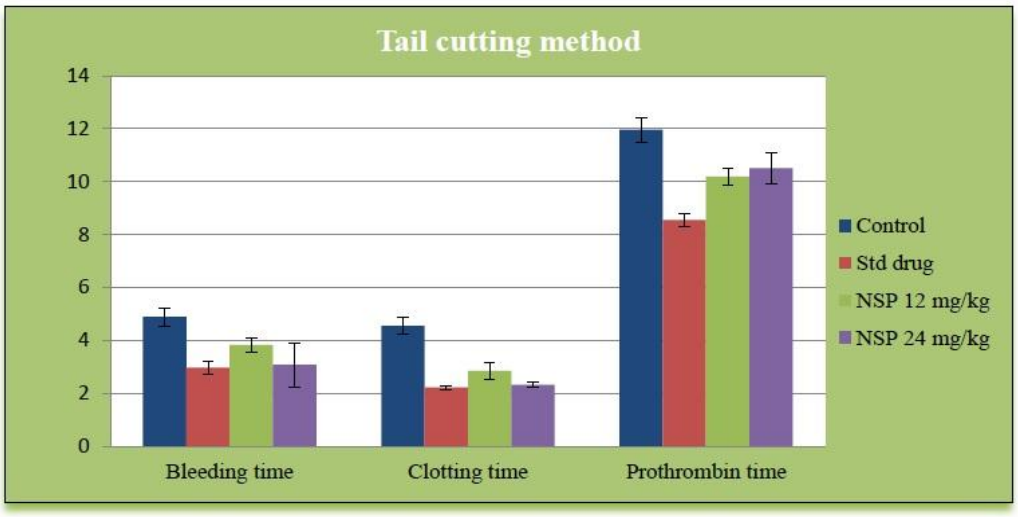

Figure 1: The mean value of Bleeding time, Clotting time and Prothrombin time of control and treated groups of Wistar albino rats exposed to Naaga Sangu Parpam.

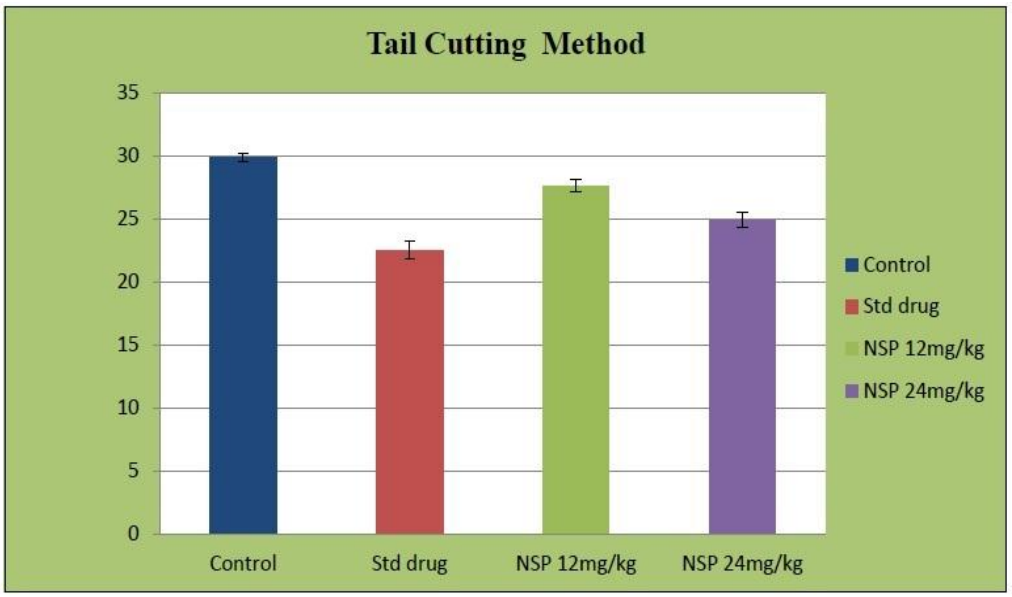

Figure 2: The mean value of Thromboplastin time of control and treated groups of Wistar albino rats exposed to Naaga Sangu Parpam.

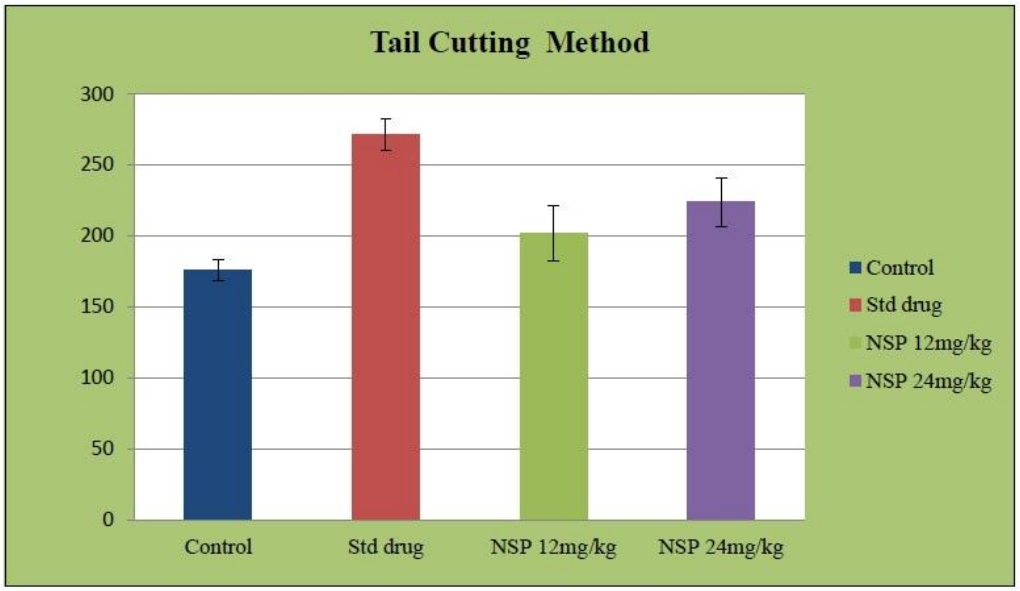

Figure 3: The mean value of Fibrinogen time of control and treated groups of Wistar albino rats exposed to Naaga Sangu Parpam.

CONCLUSION

It is concluded that administration *Naaga Sangu Parpam* at both 12mg/kg and 24mg/kg exhibits significant (***P < 0.001) Styptic activity in Wistar albino rats when compared with control group.

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Conflict of interest

Nil.

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