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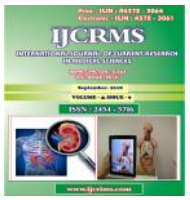

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Preclinical Investigation of Styptic (Anti-hemorrhagic) activity of Siddha Formulation *Maampisin Chooranam* in wistar rats

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Abstract

Hemorrhages are considered to be one of the potential and leading causes of death throughout the world. Clinical conditions such as menorrhagia, hemorrhoids, stroke are the most common forms of hemorrhages which inclusive of surgical interventions, traumatic injury that has been encountered by the people.Controlling hemorrhage will always remain a top priority in trauma care, and the development of materials to achieve this goal more effectively is of obvious benefit. In Allopathic system of medicine there are only limited drugs that can halt the progression of the hemorrhage. Siddha system of medicine is linked to life style and culture of the people. Siddhars lay great emphasis on strict observance of discipline(daily activities), seasonal discipline, and food regulations. According to Siddhars diseases are not only due to improper and excessive food but also due to the derangement in basic character of human being.Now herbal medicines are regarded as valuables because of its proximity to nature and trend to accept herbal medicines worldwide have been increased. A scientific investigation of medicinal plants not only demonstrates a particular type of activity which has been reported in ancient literature but also time emerges produces some unexpected activity. The main aim of the present investigation is to evaluate the styptic activity (anti hemorrhagic) of Maampisin Chooranam (MC).The results of the study reveals that treatment with MC at the dose of 100 and 200 mg/kg has shown significant decrease in bleedingtime,clotting time, prothrombin time, activated partial thromboplastin time and fibrinogen time in treated rats. From the results it was concluded that the siddha formulation MC has greater potential of preventing hemorrhage and may be used for clinical management of hemorrhage, where in further clinical investigation has been required to justify the present preclinical results.

Keywords: Hemorrhages , Siddha system , Bleeding time, Maampisin Chooranam, Clotting time, Prothrombin time, Activated partial thromboplastin time,Fibrinogen time.

1. Introduction

The process of hemostasis occurs in three phases: the vascular platelet phase, which assures primary hemostasis; activation of the coagulation cascade, which assures formation of the clot; and activation of a series of control mechanisms, which stop propagation of the clot and limit activation of the coagulation cascade to the region of endothelial rupture.

Hemostasis is the physiological process that stops bleeding at the site of an injury while maintaining normal blood flow elsewhere in the circulation. Blood loss is stopped by formation of a hemostatic plug. The endothelium in blood vessels maintains an anticoagulant surface that serves to maintain blood in its fluid state, but if the blood vessel is damaged components of the subendothelial matrix are exposed to the blood. Several of these components activate the two main processes of hemostasis to initiate formation of a blood clot, composed primarily of platelets and fibrin. This process is tightly regulated such that it is activated within seconds of an injury but must remain localized to the site of injury [1].

Menorrhagia is one of the most common bleeding manifestation in women. Menorrhagia or bleeding which is excessive in amount and duration during menstruation is one of the gynecological complaints in which out of 20-25% of women report suffering from it. One in 20 women aged 30-49 consults doctor each year with menorrhagia. Causes of the condition include disorders of the blood that affects blood clotting, pelvic infections, fibroids, endometrial polyps, endometrial hyperplasia and even pelvic cancers. If there is no obvious cause mennorhagia falls into category of Dysfunctional Uterine Bleeding (DUB). Heavy menstrual episodes may negatively affect quality of life by limiting normal activities, social life and work of female population.

Hemorrhoids are one of the most common gastrointestinal disorders seen by general practitioners. It has been estimated that they can occur at any age and can affect both men and women [2]. The natural evolution of hemorrhoids is benign in nature, but they tend to get worse over time, and therefore they should be treated as soon as it occurs. The term hemorrhoids (or piles)is used to describe the enlargement of the venous tissues of the anal region, which becomes inflamed or prolapsed [3].

The anal canal consists of three fibrovascular cushions that are supported within the anal canal by a connective tissue framework, which is important in providing a watertight seal to the anus. Hemorrhoids result from the hypertrophy of the hemorrhoidal plexus and pathological changes in the anal cushions [4].The degenerative effects of aging and regular straining during bowel movements may weaken the supporting tissues, producing a shearing force on the cushions, causing their descent and prolapse. The prolapsed cushions impair venous return, resulting in engorgement that may be further exacerbated by chronic straining during defecation, inadequate fiber intake, and conditions such as pregnancy, that raise intra-abdominal pressure. Bleeding from the engorged prolapsed hemorrhoid occurs as a result of localized mucosal trauma or inflammation, which damages the underlying blood vessels. The anal cushions of patients with hemorrhoids show significant pathological changes like abnormal venous dilatation, vascular thrombosis, degenerative process in the collagen fibers and fibroelastic tissues, distortion and rupture of the anal subepithelial muscle [6]. The symptoms associated with hemorrhoids include rectal bleeding, perianal pain, discomfort, mucous discharge, perianal itching, and irritation [7-9].

Hemorrhoids are generally classified according to their position relative to the dentate line. External hemorrhoids originate below this line and become symptomatic only when thrombosed. Internal hemorrhoids arise above the dentate line and are marked by bleeding and protrusion [10].They can be further graded according to the degree of prolapse [11,12].Internal hemorrhoids that bleed but do not prolapse are designated as Grade I hemorrhoids; those that prolapse and reduce spontaneously (with or without bleeding) are second grade hemorrhoids; prolapsed hemorrhoids requiring manual reduction are

Grade III hemorrhoids; and prolapsed hemorrhoids that cannot be reduced are Grade IV hemorrhoids.

Treatment options vary based on the degree and severity of symptoms. Management of hemorrhoids may be medical (prescribing high fiber diet, anti-motility agents, topical analgesics and corticosteroid creams for symptomatic relief, alternative/traditional medicine like oral flavonoids), non-operative (sclerotherapy, cryotherapy, rubber band ligation, infrared photocoagulation, etc.) or surgical (open, closed, or stapled hemorrhoidectomy).

The super specialty and major advantage in use of siddha medicine are “No synthetic chemicals and no side effects”. Siddha pharmacopoeia though has a wide range of drugs including medicinal herbals, minerals, metals and animal products, plant origin drugs play a significant role in most of the siddha formulations. According to the Siddha medicine various physiological and psychological functions of the body are attributed to the combination of seven elements: first is saram (plasma) responsible for growth, development and nourishment; second is senneer (blood) responsible for nourishing muscles, imparting colour and improving intellect; the third is ooun (muscle) responsible for shape of the body; fourth is kozhuppu (fatty tissue) responsible for oil balance and lubricating joints; fifth is enbu (bone) responsible for body structure and posture and movement; sixth is moolai (nerve) responsible for strength; and the last is sukilam (semen) responsible for reproduction. The treatment in Siddha medicine is aimed at keeping the three humors (Vatham, pitham, kabam) in equilibrium and maintenance of seven elements. The main aim of the present research work is to evaluate Anti-Hemorrhagic (Styptic) potential of siddha formulation Maampisin Chooranam in wistar rats.

2. Materials and Methods

2.1. Source of raw drugs:

The Required raw materials were procured from a well reputed indigenous drug shop from, Chennai, Tamil Nadu, India .All raw drugs were authenticated by respective authorities before utilizing the same for the preparing the formulation.

2.2. Ingredients

The siddha formulation *Maampisin Chooranam* comprises of the following ingredients

Nattu Maampisin (Gum of *Mangifera indica*) : 35 gm
Nelli vattral (Dried fruit of *Phyllanthus emblica*) : 35 gm
Panangkarkandu (Dried palm sugar) (*Borassus flabellifer*) : 35 gm

2.3. Formulation of Trial drug *Maampisin Chooranam* [13]

The above given drugs were purified and grinded, then sieved it by using cloth and preserved it in air tight container as mentioned in the literature.

2.4. Animal

Healthy adult Wistar albino rats were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air. A 12 light / dark cycle were maintained .Room temperature was maintained between 22 $\pm$ 2^0 C and relative humidity 50–65%. They were provided with food (Sai feeds, Bangalore, India) and water *ad libitum*. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of C.L.Baid Metha College of Pharmacy, Chennai, Tamil Nadu, India. LI/15/CLBMCP/2017.

2.5.Grouping

Animals were randomized into four groups of six animals each.

Group I rats received vehicle,
Group II rats received test drug 100mg
Group III rats received test drug 200mg
Group IV served as standard. (Tranexamic acid-TA)

The animals were administered the test drug orally and the blood sample were collected periodically for evaluation.

2.6. Determination of Blood clotting profile [14]

2.6.1. Clotting time (CT)

The tail of the animal is warmed for 1 min in water at 40°C, dried and cut at the tip with a razor blade. A 25 µl sample of capillary blood was collected into a microhematocrit glass capillary. The chronometer was started when the blood is first made contact with the glass capillary tube. The blood left to flow by gravity between the two marks of the tube, 45 mm apart, by tilting the capillary tube alternately to +60° and -60° angles with respect to the horizontal plane until blood ceased to flow (reaction end point).

2.6.2.Bleeding time (BT)

The tail of the rat is 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 started and noted when the first drop touched the circular filter paper and checked at 30 s intervals until bleeding stops.

2.6.3.Prothrombin time (PT)

0.1 ml of plasma mixed with 0.2 ml of PT reagent (calcium thromboplastin) maintain 37^{o}C, and observe the animals until formation of the fibrin clot. The time should be noted.

2.6.4.Activated Partial Thromboplastin time (APTT)

0.1 ml of plasma with 0.1mi of APTT reagent (cephalin-karolin suspension) incubated 37^{o}C for 5 minutes and then adds 0.1ml of 0.025ml cacl2 solution, until formation of the fibrin clot visually detected. The time should be noted.

2.6.5.Fibrinogen time

0.25ml of animal blood plasma add 0.05 ml of saline, and incubated 37^{o}C. After 30sec add 0.1ml of streptokinase solution, wait for 30sec, then add 0.1ml of bovine thrombin added. Start the stopwatch note at which time the fibrinogen clot formed.

3. Results

3.1.Effect of *Maampisin Chooranam* on Blood clotting profile in wistar rats

The results analysis of the blood clotting profile of control and drug treated rats has revealed that treatment with *Maampisin Chooranam* at the dose of 100 and 200 mg/kg possess significant reduction in clotting profile time such as bleeding time ,clotting time, prothrombin time, activated partial thromboplastin time and fibrinogen time when compare to the control rats. Similar results were observed in rats treated with standard drug.

Table 1: Effect of *Maampisin Chooranam* on Blood clotting profile in wistar rats

S.no	Groups	Bleeding time (sec)	Clotting time (sec)	Prothrombin time	Activated Thromboplastin time	Fibrinogen time
1	Control	84.32±2.22	119.4±1.24	26.26±1.44	27.12±5.22	196.4±3.22
2	Low dose	96.21±4.21	117.2±6.21	25.54±0.43	24.42±6.42	175.2±2.20
3	High dose	92.52±3.11	108.6±4.44	22.11±1.64	17.12±5.22	145.6±4.32
4	Standard	81.42±3.64	101.7±6.62	19.2±1.22	13.06±4.11	115.8±5.42

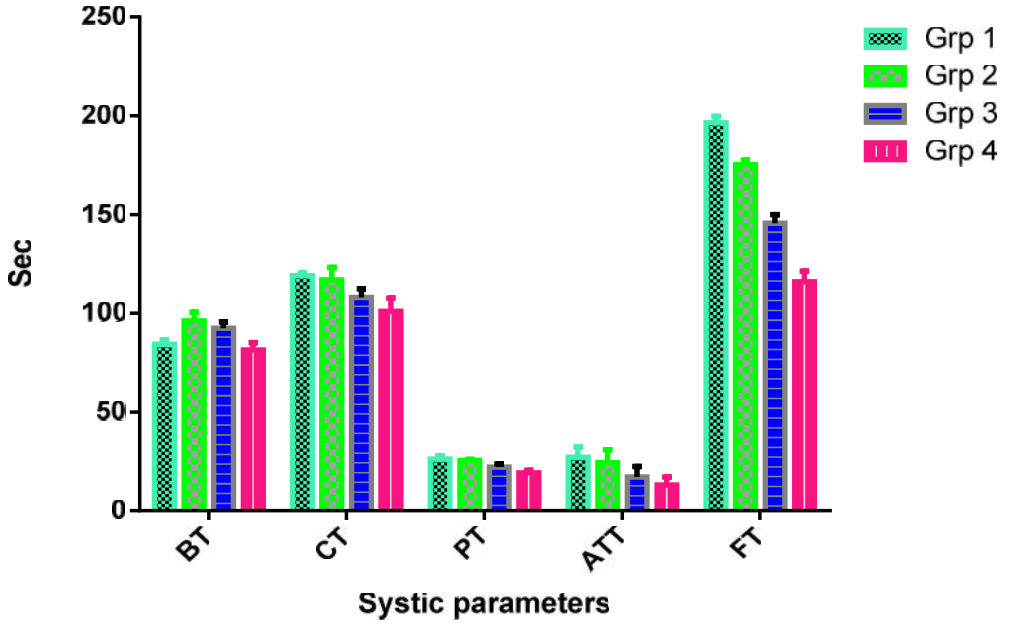

Figure 1: Result of MC treatment in Blood clotting profile in wistar rats

4. Discussion

Blood loss, while minor in every day cuts and bruises, is one of the main causes of mortality. Hemorrhage threatens the life safety of patients and the wounded in trauma care and surgical intervention. Hemorrhage is the main reason in the causes of death in 48 h after trauma, which accounts for 80% in all trauma accident [15]. Early control of hemorrhage remains the most effective strategy for treating combat casualties. Catastrophic blood loss often results in hemorrhagic shock as demonstrated in animal models [16-18], resembling human outcomes [19-21]. Therefore development of compounds to improve hemostasis and save patient's life in the trauma is of medical importance.

A number of hemostatic agents have recently been deployed to the warfront that can be used to arrest bleeding before surgical control of the source [22]. The ideal hemostatic agent would be easy to use, inexpensive, and rapidly available with no special storage requirements, non-immunogenic, versatile, and biologically inert with minimal side effects [23]. Medicinal plants are used with in a context of a traditional medicine that confronts health and illness from an integral vision.

Controlling hemorrhage will always remain a top priority in trauma care, and the development of materials to achieve this goal more effectively is of obvious benefit. In response to the changing combat and trauma casualty care, there has been an increase in efforts to develop better hemostatic agents. An ideal agent should be effective, easy to use, safe, logistically superior, and durable [24]. The results analysis of the blood clotting profile of control and drug treated rats has revealed that treatment with *Maampisin Chooranam* at the dose of 100 and 200 mg/kg possess significant reduction in clotting profile time such as bleeding time ,clotting time, prothrombin time, activated partial thromboplastin time and fibrinogen time when compare to the control rats. Similar results were observed in rats treated with standard drug.

5. Conclusion

With the current therapeutic condition of hemorrhage, an effective hemostatic drug is significant. Because hemostasis is important to save patient's life.It concluded from the data's of the present study that the siddha formulation *Maampisin Chooranam* possess significant decrease in bleeding time ,clotting time, prothrombin time, activated partial thromboplastin time and fibrinogen time dose dependently. The present findings, although

provides sufficient supporting data but it requiring confirmation by a larger trial, show that further research is needed before clinical application of this formulation.

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