



**PRE-CLINICAL EVALUATION OF ANTI-HEMORRHAGIC POTENTIAL OF
TRADITIONAL SIDDHA FORMULATION MEGARAJANGA KIRUTHAM IN ASPIRIN
INDUCED BLEEDING TIME PROLONGATION IN WISTAR RATS**

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ABSTRACT

Menorrhagia or hypermenorrhea is defined as menstrual blood loss of more than 80 ml per cycle or longer than 7 days or both of them. Menorrhagia can be associated with fibroids, endometriosis, adenomyosis, cervical or endometrial malignance, intrauterine devices, or pelvic infection. Menorrhagia diagnosis and blood loss can be identified by making use of various methods such as women's own statements, menstruation duration, the number of sanitary pads used in each menstruation, weight of sanitary. Generally, the medical practitioner will prescribe one of three common types of medications. The options include: nonsteroidal anti-inflammatory medications, oral contraceptives, and medicated intrauterine devices. A variety of different herbal supplements have been used to treat menorrhagia. The goals of alternative treatments of menorrhagia are the same as the goals of conventional treatment: control the bleeding, prevent and treat anemia, and restore an acceptable menstrual pattern. Siddha therapy pioneers the remedies for management of uterine disorders like menorrhagia, still now there is no proper documentary evidence claiming the therapeutic outcomes of traditional siddha medicine, Hence the main aim of the present investigation is to evaluate the anti- menorrhagic potential of novel siddha formulation megarajanga kirutham (MRK) in aspirin induced bleeding time prolongation in wistar rats. Results of the study reflects that MRK at both the dose level significantly shorten the bleeding time and also effective reduced the clotting and prothrombin time in treated rats. In conclusion medications like megarajanga kirutham serves as excellent remedy for manging menorrhagia at clinical level in near future.

KEY WORDS: *Menorrhagia, Siddha therapy, Megarajanga kirutham, Aspirin, Bleeding time, Clotting time, Prothrombin time*

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1. Introduction

Menorrhagia is defined as a menstrual blood loss of 80 mL per month. Population studies have shown that this amount of blood loss occurs in 10% of the population [1]; yet nearly a third of all women consider their menstruation to be excessive [2]. This symptom thus creates a substantial workload for health services.

Menorrhagia can be associated with both ovulatory and anovulatory ovarian cycles. It is important to distinguish the menstrual consequences of each cycle. Ovulatory ovarian cycles give rise to regular menstrual cycles whereas anovulatory cycles result in irregular menstruation or, extremely, amenorrhoea. Both ovulatory and anovulatory cycles can give rise to excessive menstrual loss in the absence of any other abnormality; so called dysfunctional uterine bleeding. Other disorders may be associated with excessive loss, for example, fibroids and adenomyosis, but the association may not always be causal. Endocrine disorders do not cause excessive menstrual loss, with the exception of the endocrine consequences of anovulation. Equally, except in selected populations, haemostatic disorders are rare causes of menorrhagia despite suggestions to the contrary [3].

Several methods such as drugs (including oral contraceptive pills (OCP) consumption and nonsteroidal anti-inflammatory drugs [NSAIDs]), nonpharmacological treatments (including exercise, heat therapy, acupuncture, and dietary supplements (vitamins E, B, C, and Ca, Mg) and medicinal herbal have been used for treatment of excess menstrual bleeding [4,5]. Synthetic drugs, especially in long-term administration, have side effects. Nausea, stomach irritation, ulcers, renal papillary necrosis, and decreased renal blood flow are the side effects of prostaglandin synthesis inhibitors [6]. On the other hand, most of the young women have no tendency to use hormones to reduce pain. Today, regarding the effects of chemical drugs and the usage of herbal medicine, as well as alternative and complementary therapies in treatment of diseases, many interested researchers have been drawn to this area.

Siddha system of medicine become potential alternate therapeutic source for management of uterine disorders like menorrhagia. Medicine made of herbals claims the advantage of unique phytotherapeutic composition in adequately strengthening the uterine

muscles and reclaims the rejuvenation on normalizing the blood clotting physiology. Further siddha medication offers to become a good remedy of acting as anti-anemic agents. Today in herbal medicine, numerous benefits have been found. Herbal medicines reduce the level of prostaglandins, have nitric oxide modulation effects, increase the levels of beta-endorphin, block calcium channels and improve circulation; thus, are effective in the treatment of dysmenorrhoea [7-9]. The main aim of the present investigation is to evaluate the anti-menorrhagic potential of novel siddha formulation megarajanga kirutham (MRK) in aspirin induced bleeding time prolongation in wistar rats.

2. Materials and Methods

2.1. Experimental Animals

Healthy adult Wistar albino rats of either sex weighing between 200-220 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air handling unit. A 12 light / dark cycle were maintained. Room temperature was maintained between 22 – 28 °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 Sathyabama Institute of science and technology, Chennai, Tamil Nadu, India. The IAEC approval number: SU/CLATR/IAEC/XIII/129/2019.

2.2. Experimental Methodology [10]

The animals were grouped into four groups of 6 animals each. Group I (Control group) -received normal saline 5ml/kg, Group II – Aspirin control received 5mg/kg of aspirin, p.o. for 35 days. Group III - Received Aspirin (5mg/kg) for 21 days and then treated with 200mg/kg MRK ,p.o one hour prior to Aspirin administration from day 22 to 35. Group III - Received Aspirin (5mg/kg) for 21 days and then treated with 400mg/kg MRK ,p.o one hour prior to Aspirin administration from day 22 to 35.

2.3. Bleeding time prolongation in rats [11]

Oral administration of Aspirin (5mg/kg),p.o for 21 days will cause significant change in the mean bleeding and clotting times

2.4. Determination of Bleeding Time [12]

At the end of 35th day bleeding time was evaluated. The tail of the rat was warmed for 1min in water at 40°C and then dried. A small cut was made in tail tip with a scalpel. Bleeding time start and was noted when the first drop touched the circular filter paper and checked at 15 sec intervals until bleeding stops.

2.5. Determination of Clotting Time

Clotting time was determined by capillary tube method. Capillary tube was filled with rat blood collected through retro orbital sinus puncture. Tube was broken in to small piece for every 15 sec. As soon as threads of fibrin were noticed, the stopwatch was stopped and the time recorded as the clotting time for that particular rat.

2.6. Prothrombin time (PT)

0.1 ml of plasma was mixed with 0.2 ml of PT reagent (Calcium thromboplastin) and then the reaction mixture was incubated at 37°C, and was absorbed until formation of the fibrin clot.

3. Results

3.1. Result Analysis on Effect of MRK on Aspirin induced bleeding time, Clotting time and Prothrombin time prolongation in rats

Present investigation clearly signifies that rats belongs to group II (Aspirin) pronounce significant increase in bleeding time when compared to that of the control, whereas treatment with MRK at both the dose level reveals promising decrease in the bleeding time of the experimental animals.

Clotting time analysis is one of the core parameters to elucidate the efficacy of the test drug. Aspirin induced rats provokes significantly increased level of clotting time in comparison with control group. Treatment with MRK at both the dose level reveals improved clotting time profile by marginal decrease in clotting time of treated rats.

Prothrombin time is the viable index on modifying potential of coagulative agent. The laboratory assessment of prothrombin time (PT) has been routinely used to determine a patient's coagulation status and inform therapy. In the present investigation Aspirin alone treated rats shown high index of prothrombin time when compared with control rats. Treatment with MRK at both the dose level reveals

significant declination in prothrombin time almost to that of the normal base line.

Table 1: Effect of MRK on Aspirin induced bleeding time, Clotting time and Prothrombin time prolongation in rats.

Treatment	Bleeding Time in Sec	Clotting Time in Sec	Prothrombin time in Sec
Control	157.3 ± 32.62	127 ± 5.86	8.5 ± 2.1
Aspirin - 5mg/kg	420.2 ± 19.5	413.3 ± 17.2	20.17 ± 3.06
Aspirin + 200mg/kg MRK	351.3 ± 15.7	347 ± 22.2	17.6 ± 2.5
Aspirin + 400mg/kg MRK	272.8 ± 17.2	299 ± 12.5	12 ± 3.5

Values represent mean ± SEM of 6 experimental animals.

4. Discussion

Excessive menstrual loss in regular menstrual cycles is the most common clinical presentation. Such patients ovulate regularly. Laboratory based research has shown that several abnormalities can occur in the endometrium of women with this problem—for example, increased fibrinolytic activity [13] and increased production of prostaglandins [14].

The estimated annual direct cost associated with heavy menstrual bleeding was 1 billion dollars while the indirect costs could be as high as 12 billion dollars because of the impact on work (days lost) and quality of life for the woman. These figures do not account for intangible costs and productivity loss due to absenteeism. Iron deficiency anemia with its symptoms of fatigue, weakness, pallor, and dizziness is a major coexisting medical problem. In traditional herbal medicine, uterine tone determines the ease of menstrual flow, therefore, if the uterus is hypotonic, there may be heavy bleeding. Improving uterine tone may normalize and regulate menstrual bleeding. Tonic herbs can be used in combination formulations for weeks and months to bring results. Life root, also known as ragwort, has been used as a female regulator in herbal medicine for conditions such as menstrual cramps, menorrhagia, and suppressed menstruation [15].

There are a number of medical and surgical alternatives available for the treatment of heavy menstrual bleeding. These include the levonorgestrel-releasing intrauterine system (LNG-IUS), oral progestins, oral contraceptives, danazol, non-steroidal anti-inflammatory drugs (NSAIDs), and anti-

fibrinolytic drugs, as well as endometrial ablation and hysterectomy. Many national guidelines consider the medical alternatives a first-line treatment, especially for idiopathic menstrual bleeding [16].

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Prothrombin time is the viable index on modifying potential of coagulative agent. The laboratory assessment of prothrombin time (PT) has been routinely used to determine a patient's coagulation status and inform therapy. In the present investigation Aspirin alone treated rats shown high index of prothrombin time when compared with control rats. Treatment with MRK at both the dose level reveals significant declination in prothrombin time almost to that of the normal base line.

5. Conclusion

In conclusion results of the study reflects that MRK at both the dose level significantly shorten the bleeding time and also effective reduced the clotting and prothrombin time in treated rats. In conclusion medications like megarajanga kirutham serves as excellent remedy for manging menorrhagia at clinical level in near future.

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