

Ruhgalu Beys: Its Anti-inflammatory Properties and Response on the *Rattus norvegicus albinus* (Albino Rat)

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ABSTRACT

This paper focused on the Maldivian traditional medicine Ruhgalu Beys and its anti-inflammatory response with regards to two tests which are the Phytochemical Test and Carrageenin Induced Edema Test. After obtaining the ingredients from an expert, the individual components or ingredients of the Ruhgalu Beys (paste) and the paste itself was tested for the presence of anti-inflammatory substances: steroids, triterpenoids, flavonoids, alkaloids, saponins, glycosides, and tannins. The Phytochemical Test results showed that trace amounts of sterols, flavonoids, alkaloids, glycosides and tannins were present while triterpenes and saponins were abundant in Ruhgalu Beys. The Carrageenan Induced Edema Test carried on 10 female Sprague Dawley rats showed that the paste had no significant difference on the inflamed paw foot volume. Since the tests may have had some limitations involved further testing needs to be done to determine whether there is any actual significant anti-inflammatory effect of the traditional medicine Ruhgalu Beys.

Keywords: Anti-inflammatory, Carrageenin, Medicinal Herbs, Phytochemical, Ruhgalu Beys

INTRODUCTION

Almost about 80% of the world's population is using herbal medicine as the primary source of healthier living without knowing its advantages and disadvantages (Ekor 2013). Many processed plants are made in different forms for use in traditional treatment of various acute and chronic conditions of various ailments (Benzie & Wachtel-Galour, 2011). Herbal medicines have been widely utilized as effective remedies for the prevention and treatment of multiple health conditions for centuries by almost every known culture. The first documented records of herbal medicine use date back 5,000 years in China. Similarly, India's Ayurvedic medicine tradition is thought to be more than 5,000 years old and herbal medicines remain an essential component of its practice. Today, the populations of certain countries still depend on herbal medicines to address their healthcare needs (Rivera et al. 2013).

Medicinal plants and their secondary metabolites are progressively used in the treatment of diseases as a complementary medicine. Medicinal plants are extensively used to treat inflammation which is defense response of our body to hazardous stimuli such as allergens and or injury to the tissues. Uncontrolled inflammatory response is the main cause of a vast continuum of disorders including allergies, cardiovascular dysfunctions, metabolic syndrome, cancer, and autoimmune diseases imposing a huge economic burden on individuals and consequently on the society (Bagad et al. 2013). Through time, different cultures have come up with multiple solutions to tackle this problem. They have formulated different herbal medicines which have proved their effectiveness to treat inflammation.

One such medicine is Ruhgalu beys, a traditional medicine utilized by Maldivians widely all over the entire island nation to treat inflammation. Ruhgalu beys literally means palm stone. It is a concoction made of plant parts that is well-known throughout Maldives. Some of the ingredients used are *Terminalia chebula*, *Aloe barbadensis*, *Balsamondendron myrrha* and Linn F. seeds which are also used extensively in traditional medicines in some other cultures as well.

Due to its ethno-botanical uses *Terminalia chebula* is one of the popular remedies used in India and South-East Asian countries in treating various diseases among which inflammation is included (Chattopadhyay 2013). *Terminalia chebula* has various composition of tannins that provide effective healing agents (Saroya 2011).

Aloe barbadensis is another plant that consists of anti-inflammatory chemicals. The leaf extract of the plants has been used medicinally for several thousands of years in many cultures as it consists of many properties which include anti-inflammatory, burn wound healing effect, immunomodulatory, antimicrobial and many more (Radha & Laxmipriya, 2015). Main chemical and compound that cause anti-inflammatory response in *Aloe barbadensis* is salicylic acid (Moghaddasi & Verma, 2011).

Plants that are used in traditional medicines can be found in various habitats. For instance, *Garcinia cambogia* that is commonly known as Kodampuli or Malabar tamarind is found in the two ecosystems of India, the Western Ghats and The Himalayan foot hills (Madappa & Bopaiah, 2012). Flower extract of *Garcinia cambogia* contains the phytoconstituents such as alkaloid, carbohydrate, coumarins, proteins, phytosteroids, flavonoids, phenol, steroids and triterpenoids. Flavonoids contribute to anti-inflammatory activities. The bark of the stem contains benzophenones such as garcinol isogarcinol and contain HCA (George et al. 2015). Xanthenes which are present in

Garcinia cambogia have powerful antioxidant activity. Xanthones can help reduce inflammatory enzymes in your body (Staker 2017).

Different parts of various plants are used in traditional medicines. The aromatic resin known as myrrh (mur higazy) produced by *Balsamodendron Myrrha* which exudes spontaneously from the bark is used as an ingredient in various traditional medicines. Myrrh is used today in medicine to treat wounds, relieve painful swelling, and to treat menstrual pain due to blood stagnation. It has long been used in the Ayurveda system of medicine (Hanuš et al. 2005).

In addition to *Terminalia chebula*, *Strychnos potatorum* Linn. F seeds are also used to make Ruhgalu Beys. It is a moderate sized tree found in various regions of South Asia. According to the knowledge of Ayurveda, *Strychnos potatorum* Linn F seeds are acridalexipharmacolithotriptic and cures strangury, urinary discharges, head diseases etc. They are bitter, astringent to bowels, aphrodisiac, tonic, diuretic and good for liver, kidney complaints, gonorrhea, colic, etc. Similarly, to *Terminalia chebula* and *Aloe barbadensis*, Linn. F seeds consists of anti-inflammatory chemicals such as Saponins which was revealed by phytochemical investigations on the seeds (Kavita 2014).

The use of herbal medicinal products and supplements has increased tremendously over the past three decades with not less than 80% of people worldwide relying on them for some part of primary healthcare. Although therapies involving these agents have shown promising potential with the efficacy of a good number of herbal products clearly established, many of them remain untested and their use are either poorly monitored or not even monitored at all (Ekor 2013). In addition to this, herbal medicines are preferred over synthetic drugs by some people as they tend to have less side effects. The additional health issues that can be caused due to modern medicines can be avoided with the usage of herbal medicines hence indicating that herbal medicines are more cost effective.

Therefore, herbal medicines are often viewed as a balanced and moderate approach to healing. Individuals who use them as home remedies and over-the-counter drugs spend huge amount of money (more than billions of dollars) on herbal products. This explains in part the reason sales of herbal medicines are booming and represents a substantial proportion of the global drug market. Moreover, herbal medicine is readily available making it more affordable for everyone which could save millions of lives around the globe. The research will investigate Ruhgalu Beys a traditional medicine's anti-inflammatory property and medical potential as an alternative or complementary medicine. Its specific objectives are to: 1) determine anti-inflammatory property of individual components in Ruhgalu Beys in terms of: tannins, sterols, triterpenes, flavonoids, alkaloids, saponins, and glycosides; 2) determine the anti-inflammatory property of the aqueous medicinal extract Ruhgalu Beys in terms of: tannins, sterols, triterpenes, flavonoids, alkaloids, saponins and glycosides; and 3) determine the significant anti-inflammatory response when the medicine Ruhgalu Beys is applied on the inflamed area of laboratory rats, when compared to ones without the medicine. Contemporary medications are not readily available worldwide. Owing to this reason, people living in rural areas opt for herbal medicines to treat ailments. Ruhgalu Beys is one among such herbal medicines used to treat inflammation. It can be an alternative to synthetic medications, hence, this medication will be a readily available and affordable option for people all over the globe to treat inflammation. Nowadays, various fields carry out a myriad of research on contemporary medicine. Therefore, this will be an opportunity for researchers to go in depth into the knowledge of traditional medicines and its constituent helping them in the production of new organic contemporary medicine instead of synthetically prepared medicines. This will improve the health

of the population of the world as Ruhgalu Beys, being an organic medicine will have insignificant side effects compared to synthetic medications. Moreover, this will benefit students as this is an unexploited field of study which can be explored in various ways that will benefit the society. Students can further enhance their knowledge and become doctors specialized in traditional medicine.

MATERIALS AND METHODS

Research Design

The research is considered to follow an exploratory and experimental research method where the anti-inflammatory properties and the related constituents of the Maldivian traditional medicine Ruhgalu Beys are determined using a phytochemical analysis test and a Carrageenin induced hypersensitivity test carried on rats.

Sample and Sampling Technique

The ingredients used to make Ruhgalu Beys were obtained with the help of an expert in Maldivian traditional medicine. The gel inside the *Aloe barbadensis* plant leaves was collected and allowed to dry. This will harden the gel and it will turn black. *Balsamodendron myrrha*'s myrrha, a resin that exudes from the bark of the plant were collected and allowed to harden. Both the *Terminalia chebula* and the *Strychnos potatorum* plant's seeds were collected. Same as in *Balsamodendron myrrha* plant, the *Garcinia cambogia* plant's resin was collected from the bark of the plant and allowed to solidify.

Aloe barbadensis, *Balsamodendron myrrha* and *Garcinia cambogia* plants hardened resin together with the seeds of *Terminalia chebula* and *Strychnos potatorum* were grated in a traditional grater with egg white to form the medicinal aqueous extract. These ingredients were grated using hands with gloves on. The prepared medicinal extract, *Ruhgalu Beys* which is composed of all the ingredients mentioned above was submitted and analyzed for the presence of steroids, triterpenoids, flavonoids, alkaloids, saponins, glycosides, and tannins at the Standards and Testing Division of Industrial Technology Development Institute (STD-ITDI) of Department of Science and Technology (DOST).

To determine the level of anti-inflammatory response, tests were carried out on animal models such as rats where the footpad was injected. For experimentation 10 female Sprague Dawley rats weighing 145-178 grams were tested for inflammatory response by the STD-ITDI of DOST.

Data Gathering Procedure

After the approval of the forms of request and clearance for ethics from DOST, the results of the tests were collected from the DOST research institute. The procedures of the tests implemented by the DOST were documented.

The medicinal aqueous extract (paste) which composed of all the ingredients was submitted to the STD-ITDI DOST to be analyzed for the presence of saponins, tannins, glycosides, flavonoids, alkaloids, sterols and triterpenes using Phytochemical Test and Carrageenin Induced Edema Test.

Data Analysis

The research utilizes the descriptive statistics in the analysis of the phytochemical test and would use the t-test method in the analysis of the carrageenin induced rats test. The average mean of the foot volume difference for the negative control, positive control and the paste is 0.14500, 0.06883, and 0.17625 respectively.

RESULTS AND DISCUSSION

Anti-inflammatory response is the body's response to the various changes that tissues undergoes when it becomes inflamed. A carrageenin induced edema test and a phytochemical test was carried out to determine the presence or absence of 7 specific anti-inflammatory chemicals in Ruhgalu Beys and in individual components used to make Ruhgalu Beys. Based on the results obtained, the phytochemical test showed promising results.

Table 1.0. Phytochemical Test Result (Evans & Trease, 2002)

Sample Name	<i>Garcinia cambogia</i> Resin	<i>Balsomodendron myrrha</i> Crystals	<i>Aloe barbadensis</i> Crystals	<i>Terminalia chebula</i> Seed	<i>Strychnos potatorum</i> Seed	Sample Paste
Description & Identification	About 100 g ground, solid, orange resin, unmarked	About 100 g ground, solid, brownish plant material, unmarked	About 100 g ground, black plant material, unmarked	About 100 g ground, solid, brown plant material, unmarked	About 100 g light brown, spherical shaped seeds, unmarked	About 20 g orange plant paste, unmarked
Sterols	(-)	(+++)	(+)	(+)	(+++)	(+)
Triterpenes	(+++)	(+++)	(+++)	(++)	(+++)	(+++)
Flavonoids	(+)	(+)	(+++)	(++)	(++)	(+)
Alkaloids	(+)	(+++)	(++)	(+)	(++)	(+)
Saponins	(+++)	(++)	(++)	(+++)	(+++)	(+++)
Glycosides	(++)	(+)	(+++)	(+++)	(+)	(+)
Tannins	(+)	(+)	(+++)	(+++)	(+)	(+)

Legend: (+) Traces (++) Moderate (+++) Abundant (-) Absence

The Phytochemical Test results in Table 1.0 showed that in *Garcinia cambogia* resin abundant levels of triterpenes and saponins were present and glycosides were at moderate levels. However, flavonoids, alkaloids and tannins were found to be present in traces amount (George et al. 2015). In contrast in *Garcinia cambogia* resin, it was found out that Sterols were absent. This shows that in terms of all the phytochemicals tested except sterols, anti-inflammatory property is present in *Garcinia cambogia* Resin. A research done in 2015 revealed that the crude extract or constituents from the *Garcinia cambogia* plant exerted anti-inflammatory activities in vitro and in vivo models (Semwal et al. 2015). Moreover, phytochemical studies of various plant parts revealed the presence of mainly xanthenes (e.g. carbogiol) which contribute to the anti-inflammatory property of *Garcinia cambogia* by reducing inflammatory enzymes in the body (Staker 2017).

The presence of sterols, triterpenes, and alkaloids in abundant levels, saponins in moderate levels and flavonoids, glycosides and tannins in traces amount in Table 1.0 shows that in *Balsomodendron myrrha* crystals, anti-inflammatory property is present. The results of several studies confirm anti-inflammatory and antiarthritic activities of *Balsomodendron myrrha*. One of the studies showed that 50 percent aqueous methanolic extract of *Balsomodendron myrrha* was found to exhibit an anti-inflammatory effect on adjuvant-induced air pouch granuloma in mice. The methanolic extract inhibited nitric oxide production in lipopolysaccharide activated mouse peritoneal macrophages.

Moreover, a crystalline steroid was isolated from the petroleum ether extract and tested in rats for inhibition of inflammation induced by Freund's adjuvant. It inhibited the full development of the primary lesions in adjuvant arthritis and reduced the severity of secondary lesions as compared with the untreated control group (Sarup et al. 2015). In the case of *Aloe barbadensis* crystals, the Table 1.0 shows that triterpenes, flavonoids, glycosides and tannins were present in abundant levels. Alkaloids and saponins were present in moderate levels. However, sterols were present only in traces amount. This shows that in *Aloe barbadensis* crystals, anti-inflammatory property is present in terms of all the phytochemicals tested. Aloe vera (*Aloe barbadensis*) gel has strong immunomodulatory activity wherein it down regulates lipopolysaccharide-induced inflammatory cytokine production and expression of NLRP3 (NACHT, LRR, and PYD domain-containing protein 3) inflammasome in human macrophages. *Aloe vera* could inhibit the inflammatory process following burn injury, as characterized by the reduction of leukocyte adhesion, as well as proinflammatory cytokines (Liu et al. 2012). *Aloe vera* directly inhibits the cyclooxygenase pathway and reduces prostaglandin E2 production, which plays an important role in inflammation. Aloe also contains anthraquinones and chromone in the inner gel, which possess strong anti-inflammatory effects as shown in murine macrophages (Babae et al. 2012).

The phytochemical test done on *Terminalia chebula* seed in Table 1.0 shows that saponins, glycosides and tannins were present in abundant levels. While triterpenes and flavonoids were present in moderate levels. Only sterols and alkaloids were found in traces amount. This shows that in terms of all the phytochemicals tested, anti-inflammatory property is present in *Terminalia chebula* seed. The aqueous extract of dried fruit of *Terminalia chebula* and its immature seeds have the chemical chebulagic acid the phytochemicals mentioned above which provides anti-inflammatory properties. The anti-inflammatory property is initiated by inhibiting inducible nitric oxide synthesis or suppressing the onset and progression of collagen inducing arthritis (Chattopadhyay 2013).

Table 1.0 also shows that in *Strychnos potatorum* seed, sterols, triterpenes and saponins were present in abundant levels. While flavonoids and alkaloids were present in moderate levels. However, glycosides and tannins were present only in traces amount. This shows that in *Strychnos potatorum* seed anti-inflammatory property is present in terms of the above-mentioned phytochemicals. The anti-inflammatory actions of polyphenols and the above-mentioned phytochemicals are associated with their stimulation of peroxisome proliferator activated receptor gamma. It controls lipid uptake and fat cell synthesis. Increased expression of this gene transcription factor also inhibits the activation of nuclear factor kappa B which is the master switch for turning on the innate inflammatory response (Cassidy et al. 2013).

Lastly the Table 1.0, shows that the sample of Ruhgalu Beys used in the phytochemical test and tested for the above-mentioned tests for each anti-inflammatory chemical show that, triterpenes and saponins were present abundantly in the sample of Ruhgalu Beys. Only traces of other chemicals; sterols, flavonoids, alkaloids, glycosides and tannins were present in the sample of Ruhgalu Beys. This shows that in terms of triterpenes and saponins, anti-inflammatory property is present in *Ruhgalu Beys* at a higher level than the other anti-inflammatory chemicals. Triterpenes was found by Bachhav et al. (2011) to confer nitric oxide releasing mechanism, significantly decreasing the level of plasma nitrate/nitrite. In various studies, triterpenes were also found to inhibit COX-2 enzyme and Th1 responses, which can reduce the risk of inflammation (Han & Bakovic, 2015). Saponins significantly inhibit nitrite production by 50% (Borgia et al. 2008). Moreover, crude saponins extracts tested have significant anti-inflammatory activity that might be mediated through

the inhibition of the release and synthesis of the agents that produce inflammations (Hassan et al. 2012).

Table 2.0. Carrageenin induced edema method result

Test Sample	Number of test animals	Percent protection against edema
Indomethacin (Positive Control)	3	52.87
Paste (Ruhgalu beys)	4	0

According to the results obtained, Table 2.0 shows the percent protection against edema for both the test samples of indomethacin and the paste. The percent protection for indomethacin is 52.87% while for the paste Ruhgalu Beys percent protection is 0%. The carrageenin induced edema test was carried on rats belonging to the species *Rattus novergicus albinus* that are being used in various other test models for drug testing. The carrageenin is injected onto the paw of rats and various drugs are orally ingested, injected or topically applied onto the surface of the inflamed area.

Carrageenin being an inflammation inducing agent used to create a local inflammation at the injected area, shows the five cardinal signs of inflammation which are: hypersensitivity, redness, swelling, heat, and loss of function. These cardinal signs are applicable to all situations and can be used to distinguish when an inflammation has occurred. Various tests have been carried on rat models among which include oral drugs taken by rats while the foot is injected with carrageenin. The experiment shows how oral doses taken by the rats have the effects of its anti-inflammatory agents (Winter et al. 1962).

Anti-inflammatory activity against carrageenin induced paw in rats for the plant *Garcinia cambogia* also determined its response. The experiment showed appreciable levels of anti-inflammatory activity for the plant *Garcinia cambogia* (Prasanth et al. 2013).

Table 2.1. Summary of anti-inflammatory test in Sprague Dawley Rats using Carrageenin induced edema in rats methods

Group Number	Test Sample	No. of animals	Foot volume difference	Average of foot volume difference	Percent (%) Protection
I	Negative Control	3	0.1550	0.14500	
			0.1000		
			0.1800		
II	Indomethacin (Positive Control)	3	0.0600	0.06883	52.87
			0.0400		
			0.1050		
III	Paste (Ruhgalu beys)	4	0.1950	0.17625	-21.55
			0.0750		
			0.2000		
			0.2350		

Like in another test showed the Anti-inflammatory activity of crude Saponins extracts from five Nigerian medicinal plants and its effects on paw of rats injected with carrageenin (Hassan et al. 2012). Since rat models are being used as a measure to determine the anti-inflammatory response of various drugs, the experiment conducted is also used to determine the response of the traditional medicine as an anti-inflammatory agent. Table 2.1 shows the foot volume difference with regards to each test sample against the negative control and the percent protection against edema. The percent protection for indomethacin is 52.87% while for the paste Ruhgalu Beys percent protection is -21.55% The table also shows how the number of animals used in each sample with the foot volume difference for each animal tested is used in the calculation of the average foot volume difference. The results show that the average foot volume difference of the negative control group is 0.14500 while for the positive control group has a value of 0.06883 and the experimental group has a value of 0.17625.

When the carrageenin induced edema, test was carried on the rats, it showed the effects of the substances applied onto the paw for the positive control, negative control and the experimental group. The results of the experimental group did not give a significant difference for the rat foot volume even though the phytochemical analysis indicated that there were a lot of anti-inflammatory substances present that can provide a positive response.

Among the experimental group only one-foot volume difference of 0.0750 gave a value close to the foot volume difference of the positive control group. Though this value was close to the positive

control group foot volume difference, it did not provide a significant effect on the average when calculated. The difference seen can be due to limitations since the phytochemical results have shown that the paste or traditional medicine possess high levels of anti-inflammatory substances.

According to the table 2.1 the value range of average foot volume difference between experimental group and positive control group were too large. If the anti-inflammatory response of the traditional medicine were to be effective it should be a value that would be close to the average foot volume difference of the positive control group seen in table 2.1. It can also be a value lower than 0.06883 which is the average foot volume difference for the positive control group. That would be a good indication of its effectiveness as an anti-inflammatory agent and as a good alternate to the commercially used indomethacin.

CONCLUSIONS

Therefore, even though promising results were obtained in the phytochemical analysis test, the carrageenin induced edema test turned out to be negative. The anti-inflammatory property of individual components in Ruhgalu Beys shows that, in terms of: 1) Tannins – an abundant level of tannins was present in *Aloe barbadensis* crystals and *Terminalia chebula* seeds. It was observed in trace amounts in the rest of the ingredients; 2) Sterols- an abundant level of sterols was present in *Balsamodendron myrrha* crystals and *Strychnos potatorum* seed. The rest of the ingredients had trace amounts of sterol; 3) Triterpenes- an abundant level of triterpenes was present in *Garcinia cambogia* resin, *Balsamodendron myrrha* crystals, *Aloe barbadensis* crystals and *Strychnos potatorum* seed. A moderate amount could be found in *Terminalia chebula* seeds; 4) Flavonoids- an abundant level of flavonoids was present in *Aloe barbadensis* crystals and a moderate amount in *Terminalia chebula* seeds and *Strychnos potatorum* seed. The rest had only trace amount of flavonoids; 5) Alkaloids- an abundant level of alkaloids was present in *Balsamodendron myrrha* crystals and a moderate amount was present in *Aloe barbadensis* crystals and *Strychnos potatorum* seed; 6) Saponins- an abundant level of saponins was present in *Garcinia cambogia* resin, *Strychnos potatorum* seed and *Terminalia chebula* seeds. A moderate amount was present in *Balsamodendron myrrha* crystals and *Aloe barbadensis* crystals; and 7) Glycosides- an abundant level of glycosides was present in *Aloe barbadensis* crystals and *Terminalia chebula* seeds. *Garcinia cambogia* resin had Sterols in moderate amount.

Anti-inflammatory property of the aqueous medicinal extract Ruhgalu Beys in terms of: 2.1) Tannins- tannins was present in trace amounts; 2.2) Sterols- sterols was present in trace amounts; 2.3) Triterpenes- an abundant level of triterpenes was present in Ruhgalu Beys; 2.4) Flavonoids- flavonoids was present in trace amounts; 2.5) Alkaloids- alkaloids was present in trace amounts; 2.6) Saponins- an abundant level of saponins was present in Ruhgalu Beys; and 2.7) Glycosides- glycosides was present in trace amounts.

According to the result of Carrageenin Induced Edema experiment, there is no significant anti-inflammatory response when Ruhgalu Beys was applied on to the inflamed area of laboratory rats. The ingredients that are used to make Ruhgalu Beys has significant amount of constituents that contribute to anti-inflammation. Abundant amount of triterpenes and saponins were found in nearly all the ingredients. Triterpenes and saponins inhibit effects on the production of pro-inflammatory mediators such as COX-2 expression and induce the production of anti-inflammatory cytokines. Other constituents that contribute to anti-inflammation were also found in considerable amount. Hence, each of the ingredient used to make Ruhgalu Beys possess anti-inflammatory properties.

These individual ingredients can be used to treat inflammation due to the presence of the anti-inflammatory components. The natural ingredients reduce the risk of allergy and overdose, so people can use it without restrictions.

Even though Carrageenin Induced Edema test indicated that there is no significant effect of Ruhgalu Beys on the paws of the rats, the Phytochemical Test proved that Ruhgalu Beys itself has copious amount of anti-inflammatory constituents. Therefore, Ruhgalu Beys can be used effectively to treat inflammation. Ruhgalu Beys can be an organic alternative to synthetic medicines used to treat inflammation. Moreover, there are no chemical preservatives added to it. This implies that everyone irrespective of age or certain health conditions can use Ruhgalu Beys.

RECOMMENDATIONS

The results of the phytochemical analysis test can be interpreted in a manner which can show the exact composition of the present constituents and it can be denoted as a percentage, hence giving the degree of anti-inflammatory property of each individual component. The experimental procedure of Carrageenin Induced Edema contradicted the traditional application of the medicine. Traditionally, the paste is applied as a thick layer on a cotton that is placed on the inflamed area. In the test procedure, unstated quantity of the concoction was applied. In contrast to this, they should have applied a measured quantity of thick layer of the concoction on a cotton pad placed on the inflamed area so that the paste can stay put and form a cast. During the experimental procedure of Carrageenin Induced Edema, the foot volume was measured 30 minutes after the topical administration of Ruhgalu Beys. This is an insufficient time for the medicine to show its effectiveness. To give the paste, ample time to settle and harden, the foot volume of rat paw can be measured at an interval of 30 minutes for 5 hours. This will give a vivid insight of the progression of the test. A thick layer of Ruhgalu Beys takes considerable time to solidify. Carrageenin results in an acute swelling that becomes maximal 3-5 hours after injection and subsides by 24 hours. Thus, Complete Freund's adjuvant, an alternative to Carrageenin induced Edema, can be utilised. Subcutaneous injection of complete Freund's adjuvant in saline induces a more prolonged swelling that becomes maximal at 24 hours and persists for at least 7 days. The extended time bought by Complete Freund's adjuvant gives adequate time for the concoction to harden and form orthopedic cast. As an alternative of Carrageenin Induced Edema test, COX1 and COX2 test can be performed. Cyclooxygenase (COX), officially known as prostaglandin-endoperoxide synthase (PTGS), is an enzyme that is responsible for formation of prostanoids, including thromboxane and prostaglandins such as prostacyclin. The two isozymes found in humans, PTGS1 and PTGS2, are frequently called COX-1 and COX-2. The main COX inhibitors are the non-steroidal anti-inflammatory drugs (NSAIDs). The resulting inhibition of prostaglandin and thromboxane synthesis has the effect of reduced inflammation, as well as antipyretic, antithrombotic and analgesic effects. COX-2 is usually specific to inflamed tissue. However, COX1 and COX2 method is expensive compared to Carrageenin Induced Edema test. Furthermore, an experiment to find out the toxicity level of Ruhgalu Beys can be carried out to find out if it causes any harm or side effects to the human body when applied.

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