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In vivo anti-inflammatory and *in vitro* antioxidant activities of a Thai traditional formula, Rid-si-duang-ma-ha-kan, for hemorrhoid treatment

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Abstract

Rid-si-duang-ma-ha-kan (RSDM), a Thai traditional formula, has been used for hemorrhoids treatment in Thai hospitals for many years but evidence is limited. This formula consists of twenty two plants in equal proportions. The formula was extracted with 80% ethanol and concentrated to dryness under pressure and controlled temperature. The crude ethanol extract in various concentrations (25, 50, 100 mg/kg) were tested for anti-inflammatory activity in cotton pellet-induced granuloma in rats. Antioxidant capacity and total phenolic contents of the RSDM formula and its component plants were also evaluated using DPPH, lipid peroxidation (TBARS), and Folin–Ciocalteu methods. It was found that the extract of the RSDM formula at 50 mg/kg, per oral, showed significant inhibitory effect ($p < 0.05$) on granuloma formation comparable to indomethacin, a standard non-steroidal anti-inflammatory drug (5 mg/kg). Among all the component plants extracts, *Terminalia chebula* Retz., *Cinnamomum bejolghota* (Buch.–Ham.) Sweet and *Cinnamomum verum* J. Presl exhibited more potent DPPH scavenging activity (IC_{50} = 4.4, 5.2, and 8.0 µg/ml, respectively) than that of Trolox and rutin (IC_{50} = 8.9 and 22.9 µg/ml, respectively). In the lipid peroxidation test, *Myristica fragrans* Houtt. (seed), *Myristica fragrans* Houtt. (aril), *Terminalia chebula*, *Cinnamomum bejolghota* and *Zingiber officinale* Roscoe showed stronger activity (IC_{50} = 1.3, 1.7, 1.8, 2.1, and 2.7 µg/ml, respectively) than that of Trolox and rutin (IC_{50} = 4.1 and 68.7, respectively). Furthermore, *Terminalia chebula* had the highest total phenolic content (34.3 mg GAE/g), followed by *Cinnamomum bejolghota* (27.1 mg GAE/g) and *Cinnamomum verum* (21.4 mg GAE/g), respectively.

Keyword: Rid-si-duang-ma-ha-kan, Anti-inflammatory, Antioxidant activity, Phenolic content, Hemorrhoids, Traditional medicine

1. INTRODUCTION

Hemorrhoids are one of the most common rectal disorders characterized by the presence of swollen and inflamed veins in the rectum and anus. They often result from applying too much pressure to the veins around the lower rectum and anal area[1]. Other factors include pregnancy, aging, chronic constipation and less frequently diarrhea. Although hemorrhoids are rarely serious, they produce uncomfortable symptoms such as pain, itching, bleeding, or thrombosis[2].

The pathological changes of the anal cushions of patients with hemorrhoid include abnormal venous dilatation, vascular thrombosis, degeneration of collagen fibers and fibroelastic

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tissues, distortion and rupture of the anal subepithelial muscle[3]. In one-hundred surgical specimens of hemorrhoidectomies, the histologic investigation demonstrated a severe inflammatory reaction that especially affected the blood vessel wall and conjunctive tissue[4].

Inflammation, which constitutes a part of the acute response, results in a coordinated influx of neutrophils at the anal site. These cells release inflammatory substances and free radicals[5]. Thus, the hemorrhoid site is rich in both oxygen and nitrogen reactive species leading to lipid peroxidation, DNA damage, and enzyme inactivation, including free-radical scavenger enzymes[6]. Evidence for the role of oxidants in the pathogenesis of many diseases suggests that antioxidants may help to maintain a balance in the body and may be of therapeutic use in these conditions[7].

Rid-si-duang-ma-ha-kan (RSDM), a Thai traditional preparation, has been used for the treatment of hemorrhoids in Thai hospitals for many years. It is one of the herbal formulas included in the List of Herbal Medicinal Products by Ministry of Public Health of Thailand (2011). The RSDM formula constitutes a mixture of 22 plant species, in the equal proportion, as shown in Table 1. However, there is little scientific research available on its biological activity related to hemorrhoids treatment. Therefore, the aim of this study was to investigate the *in vivo* action of the RSDM formula in sub-acute inflammation animal models and *in vitro* anti-oxidant properties of the formula as well as its components.

2. MATERIALS AND METHODS

2.1. Plant materials

All 22 plant materials were purchased (100–150 g per sample) from a herbal drug store in Bangkok, Thailand. Their scientific names, local names, and medicinally used parts are detailed in Table 1. Their macroscopic characteristics were identified according to Thai herbal pharmacopeia guidelines.

Table 1. Component plants in the RSDM formula; their names, local names and parts used

Scientific names	Local names	Parts used
Anethum graveolens L.	Thian-ta-tak-ka-tan	Seed
Angelica dahurica (Hoffm.) Benth. & Hook.f.ex Franch. & Sav.	Kot-so	Root
Artemisia annua L.	Kot-chu-la-lum-pha	Aerial part
Cinnamomum bejolghota (Buch.–Ham.) Sweet	Sa-mun-wang	Bark
Cinnamomum verum J. Presl	Op-choei	Inner bark
Commiphora molmol Engl. ex. Tschirch.	Mod-yop	Oleo-gum resin
Cuminum cyminum L.	Thian-khao	Seed
Foeniculum vulgare Mill.	Thian-khao-plueak	Seed
Gonostegia pentandra (Roxb.) Miq.	Khop-cha-nang-dang	Aerial part
Lepidium sativum L.	Thian-dang	Seed
Myristica fragrans Houtt.	Dok-chan (mace)	Aril
Myristica fragrans Houtt.	Luk-chan (nutmeg)	Seed
Nigella sativa L.	Thian-dam	Seed
Picrorhiza kurroa Royle ex Benth.	Kot-kan-prao	Rhizome
Piper interruptum Opiz.	Sa-khan	Stem
Piper nigrum L.	Prik-thai (pepper)	Fruit
Piper retrofractum Vahl.	Di-pli	Fruit
Pistacia chinensis subsp. *integerrima* (J. L.Stewart ex Brandis) Rech. f.	Kot-kak-kra	Root
Pouzolzia zeylanica (L.) Benn.	Khop-cha-nang-khao	Aerial part
Terminalia chebula Retz.	Kot-phung-pla	Gall
Thuja orientalis L.	Son	Wood
Zingiber officinale Roscoe.	Khing (ginger)	Rhizome

2.2. The component plants preparation

The component plants were cleaned and dried in a hot-air oven at about 50 °C for 48 h. They were then ground into a fine powder using a laboratory-scale mill. Dried powder (50 g) of each plant was macerated with 80% ethanol for 3 days and then concentrated to dryness under pressure and controlled temperature using a rotary evaporator.

2.3. The RSDM formula preparation

All the component plants were combined in equal proportions, as recommended in traditional use, and ground into a powder. The RSDM formula powder (200 g) was macerated with 80% ethanol (500 ml) for 3 days and then concentrated to dryness under pressure and controlled temperature using a rotary evaporator.

2.4. Experimental animals

Male Sprague Dawley rats, weighing 190–220 g, were obtained from the National Laboratory Animal Centre, Nakhon Pathom, Thailand. The animals were housed in individual cages and kept in a controlled environment room (25 ± 1 ºC) under 12 h light/dark cycles for at least 1 week before the experiment. The animals had free access to water and food. The experimental protocol was approved by the Institutional Animal Care and Use Committee, Faculty of Pharmacy, Mahidol University (PYT 001/2556) prior to initiation of the experiments.

2.5. Cotton pellet-induced granuloma formation

To evaluate the anti-inflammatory activity, two methods described in other studies[8,9] were applied, using a subcutaneous implantation technique with slight modification. Two sterilized cotton pellets (20 ± 0.5 mg) were implanted subcutaneously, one on each side of the shaved back region. Different extracts of the RSDM formula were administered orally, three times daily for 14 days, beginning on the day after implantation. On the 15th day, after anesthetizing rats, the cotton pellets were removed and made free from extraneous tissues. The pellets were weighed immediately for wet weight and the percentage of granuloma inhibition was calculated.

2.6. Animal experimentation and drug treatment protocol

Rats were randomly divided into five experimental groups, each consisting of five rats and were treated as follows;

Group I: Vehicle control treated animals received 5% Tween 20

Group II: Animals administered indomethacin (5 mg/kg p.o.)

Group III: Animals received ethanolic extract of the RSDM formula 25 mg/kg p.o.

Group IV: Animals received ethanolic extract of the RSDM formula 50 mg/kg p.o.

Group V: Animals received ethanolic extract of the RSDM formula 100 mg/kg p.o.

2.7. DPPH radical scavenging activity

DPPH radical scavenging activity of the ethanolic extracts of RSDM formula and its component plants were evaluated based on a method of the previous study[10]. Briefly, 95 µl DPPH solution (0.1 mM in 80% ethanol) was mixed with 5 µl of the RSDM formula or its component plant extracts (10 mg/ml) in covered 96-well plates. Then serial dilutions were made before the plates were incubated under control conditions of room temperature and darkness for 40 min. After that absorbance of the mixtures was measured at 520 nm against blank. All the experiments were carried out in triplicate. Trolox and rutin were used as reference standards.

2.8. Lipid peroxidation

To evaluate lipid peroxidation inhibitions of the RSDM formula and its component plants extracts, a thiobarbituric acid reactive substances (TBARS) assay[11,12] was carried out with some modifications[10], using liposomal suspension from type VII folch bovine brain extract and thiobarbituric acid reactive substance. Trichloroacetic acid and 2,6-di-t-butyl-p-kresol

were also used to precipitate interfering substances. The extracts in various concentrations were tested and liposome lipid peroxidation was determined. The absorbance was measured at 540 nm. All the experiments were carried out in triplicate. Trolox and rutin were used as reference standards.

2.9. Determination of total phenolic content by Folin–Ciocalteu method

Total phenolic contents of the RSDM formula and each component plant extract were determined following the method of a previous study[13]. All the extracts were prepared in various concentrations and filtered. Gallic acid was used as a standard for plotting calibration curve and was prepared in an appropriate concentration which to be recorded for calculations. The absorbance of the resulting blue color was measured at 765 nm. The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid. The content of total phenolic compounds expressed as mg/g gallic acid equivalent (GAE) of dry extract.

2.10. Statistical analysis

All values were shown as mean ± SD of triplicate experiments in antioxidant tests, and of 5 rats in cotton pellet-induced granuloma formation test. Statistical analysis was performed using one-way analysis of variance (ANOVA). Values of $p \leq 0.05$ were considered statistically significant.

3. RESULTS

3.1. Anti-inflammatory activity against cotton pellet-induced granuloma formation

The anti-inflammatory activity of the RSDM formula extract, in various concentrations, against cotton pellet-induced granuloma formation is presented in Table 2. Values are expressed as mean ± SD (n = 5).

Table 2. The effect of the RSDM formula extract on cotton pellet-induced granuloma in rats

Group	Treatment	Granuloma wet weight (mg)	% Granuloma inhibition
I	Vehicle control	120.4 ± 3.9	–
II	Indomethacin 5 mg/kg p.o.	100.1 ± 2.0*	16.9
III	RSDM formula 25 mg/kg p.o.	95.6 ± 2.1*	20.6
IV	RSDM formula 50 mg/kg p.o.	83.7 ± 1.6*,**	30.5
V	RSDM formula 100 mg/kg p.o.	91.6 ± 3.5*	23.9

* $p < 0.05$ vs. control. ** $p < 0.05$ vs. indomethacin.

All tested samples and the reference drug showed a significant ($p < 0.05$) reduction in the weight of cotton pellet granuloma when compared with control. The reduction in the weight of cotton pellet granuloma with different doses of the RSDM extracts 25, 50, 100 mg/kg were found 20.6%, 30.5%, 23.9%, respectively. The RSDM formula extract at a dose of 50 mg/kg demonstrated the maximum granuloma inhibition of 30.5%, which was superior to that of the reference drug indomethacin (16.9%, $p < 0.05$)

Table 3. Antioxidant activities of the RSDM formula extract and its component plants extracts. Values are mean ± SD of three separate experiments (n = 3)

No.	Species	% Yield	DPPH -scavenging IC50 (µg/ml)	Lipid peroxidation IC50 (µg/ml)	Total phenolics* (mg GAE/g)
1	*Anethum graveolens*	5.3	> 200.0	4.9 ± 0.4	3.5 ± 0.2
2	*Angelica dahurica*	5.8	> 200.0	> 100.0	5.2 ± 0.2
3	*Artemisia annua*	6.7	> 200.0	> 100.0	2.8 ± 0.1
4	*Cinnamomum bejolghota*	19.1	5.2 ± 0.0	2.1 ± 0.0	27.1 ± 1.0
5	*Cinnamomum verum*	9.7	8.0 ± 0.1	10.0 ± 0.0	21.4 ± 1.0
6	*Commiphora molmol*	1.5	> 200.0	> 100.0	4.8 ± 0.3
7	*Cuminum cyminum*	6.9	167.1 ± 1.3	6.7 ± 0.1	5.1 ± 0.5
8	*Foeniculum vulgare*	9.6	> 200.0	> 100.0	1.5 ± 0.0
9	*Gonostegia pentandra*	12.8	43.2 ± 0.4	37.1 ± 0.6	14.4 ± 1.2
10	*Lepidium sativum*	6.5	168.8 ± 3.7	> 100.0	6.0 ± 0.6
11	*Myristica fragrans* (Aril)	12.3	27.7 ± 1.8	1.7 ± 0.2	12.3 ± 1.5
12	*Myristica fragrans* (Seed)	16.6	48.2 ± 1.5	1.3 ± 0.0	8.8 ± 0.5
13	*Nigella sativa*	15.0	> 200.0	77.3 ± 2.4	2.1 ± 0.1
14	*Piper interruptum*	1.3	138.7 ± 2.2	38.7 ± 0.1	7.3 ± 0.8
15	*Piper nigrum*	7.0	> 200.0	26.0 ± 0.5	1.0 ± 0.2
16	*Piper retrofractum*	3.7	> 200.0	80.9 ± 2.1	2.1 ± 0.1
17	*Picrorhiza kurroa*	22.2	176.9 ± 2.8	40.9 ± 1.0	9.6 ± 1.0
18	*Pistacia chinensis* ssp. *integerrima*	7.2	165.4 ± 3.9	39.2 ± 0.0	7.7 ± 0.4
19	*Pouzolzia zeylanica*	4.4	85.1 ± 7.6	> 100.0	7.4 ± 0.2
20	*Terminalia chebula*	37.7	4.4 ± 0.2	1.8 ± 0.0	34.3 ± 0.3
21	*Thuja orientalis*	2.9	> 200.0	16.3 ± 0.5	4.2 ± 0.5
22	*Zingiber officinale*	6.4	52.5 ± 3.3	2.7 ± 0.0	11.0 ± 1.5
23	Whole formula extract	-	70.2 ± 2.5	5.3 ± 0.0	9.3 ± 0.6
24	Trolox	-	8.9 ± 0.2	4.1 ± 0.1	ND
25	Rutin	-	22.9 ± 0.0	68.7 ± 0.5	ND

* Equivalent to gallic acid and expressed as mg GAE/g ND = not determined.

3.2. DPPH radical scavenging activity

The DPPH radical scavenging activity of ethanolic extract of the RSDM formula and of its individual components extracts is presented in Table 3. The RSDM formula extract showed DPPH free radical scavenging activity with an IC_{50} = 70.2 µg/ml. Three of the plant extracts; *T. chebula* (IC_{50} 4.4 µg/ml), *C. bejolghota* (IC_{50} 5.2 µg/ml) and *C. verum* (IC_{50} 8.0 µg/ml) exhibited more potent DPPH radical scavenging activity when compared with trolox and rutin (IC_{50} 8.9 and 22.9 µg/ml, respectively). Moreover, ten of the component plants including the RSDM formula extracts showed moderate

antioxidant activity, while the others possessed mild antioxidant activity.

3.3. Lipid peroxidation inhibition

The RSDM formula extract showed lipid peroxidation inhibition with an IC_{50} value of 5.3 µg/ml which was comparable to trolox (IC_{50} 4.1 µg/ml). Among all the component plants extracts, *M. fragrans* (seed) (IC_{50} 1.3 µg/ml), *M. fragrans* (aril) (IC_{50} 1.7 µg/ml), *T. chebula* (IC_{50} 1.8 µg/ml), *C. bejolghota* (IC_{50} 2.1 µg/ml) and *Z. officinale* (IC_{50} 2.7 µg/ml) showed potent activity when compared with trolox and rutin (IC_{50} = 4.1 and 68.7, respectively).

3.4. Total phenolic content

The total phenolic content varied widely among the different component plants extracts, ranging from 1.0 to 34.3 mg GAE/g (Table 3). The RSDM formula extract contained 9.3 mg GAE/g of total phenolic content. Among all the plant extracts, *T. chebula* had the highest total phenolic content (34.3 mg GAE/g), followed by *C. bejolghota* (27.1 mg GAE/g) and *C. verum* (21.4 mg GAE/g), whereas the lowest level was found in *P. nigrum* (1.0 mg GAE/g).

4. DISSCUSSION

Cotton pellet induced granuloma is a model of non-immunological types of inflammation and edema is mainly due to proliferative phase of chronic inflammation. The implanted material induces a host inflammatory response and stimulates the release of inflammatory mediators, which ultimately lead to granuloma formation. The increase in wet weight of the cotton pellet is defined as the transudative phase of inflammatory response[8]. In this present study, although a 50 mg/kg dose of the ethanol extract of the RSDM formula demonstrated the optimum anti-inflammatory activity by inhibiting granuloma formation, it appears that the anti-inflammatory effect of the formula extract was not strongly dose-dependent.

The RSDM formula extract has been found to reduce inflammation in the animal model tested at a starting dose of 25 mg/kg p.o. as evidenced by significantly decreased weight of cotton pellet in cotton pellet-induced granuloma in rats ($p < 0.05$ vs. control). Moreover, at the dose of 50 mg/kg p.o. the RSDM formula extract demonstrated the anti-inflammatory effect that was superior to that of indomethacin, a standard non-steroidal anti-inflammatory drug ($p < 0.05$ vs. indomethacin and control). However, at the dose of 100 mg/kg p.o, the RSDM formula started to show adverse events including diarrhea and weight loss, and its anti-inflammatory effects was lower than that of a 50 mg/kg dose of the RSDM formula.

Most of the component plants in the RSDM formula have been reported to have anti-inflammatory activity in various experimental models. For example, the ethanol extract of *A. dahurica* has *in vitro* anti-inflammatory activity via the suppression of the NF-κB pathway[14]. The water extract of *A. dahurica* also suppressed carrageenan-induced rat paw edema[15]. Artemisinin extracted from *A. annua* appeared to have anti-inflammatory properties, probably due to the inhibition of pro-inflammatory factors and mediators such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, IL-1β, and nitric oxide[16,17]. In addition, the extract of *A. annua* was shown to be a potent inhibitor of TNF-α and a strong inhibitor of PGE-2 production in activated neutrophils[18].

C. verum bark is one of the oldest herbal medicines mentioned in many traditional texts for inflammatory conditions. Its active compounds such as type-A procyanidine polyphenols (TAPP) extracted from *C. verum* (synonym *C. zeylanicum*) also showed disease-modifying potential in animal models of inflammation and arthritis[19]. The methanol extract of *F. vulgare* fruits showed significant anti-inflammatory, anti-type IV allergic and central analgesic activities. Moreover, plasma antioxidant enzyme activities, lipid peroxidation and HDL cholesterol levels were affected by administration of *F. vulgare* fruits methanol extract in rats. It is suggested that *F. vulgare* (fruit) may reduce the risk of inflammation-related diseases[20].

N. sativa, is a herb used in traditional medicine in many Middle Eastern and Asian

countries to treat a broad array of diseases. Thymoquinone, the most abundant constituent of the seed oil extract, has been shown to have anti-inflammatory effect in autoimmune encephalomyelitis induced Wistar rats[21]. It also significantly reduced the expression of cytokines such as MCP-1, TNF-α, IL-1β, COX-2 and reduced the transport of NF-κB from the cytosol to the nucleus[22]. *N. sativa* polyphenols injected to the rats intraperitoneally could inhibited paw edema in a dose dependent manner[23].

Pipers are among the important medicinal plants used in various systems of medicine. Piperine, an active compound from *P. nigrum* and *P. retrofractum* are reported to have anti-inflammatory effects via inhibition the expression of IL-6, MMP-13, and reduced the production of PGE-2. Piperine also inhibited the migration of activator protein-1 (AP-1), but not NF-κB. In rats, piperine significantly reduced the inflammatory area in an arthritis animal model[24]. The water extract of *T. chebula* fruit also caused dose-dependent inhibition of carrageenan-induced acute inflammation in rats. However, in chronic inflammation, *T. chebula* at 600 mg/kg did not reduce both transudative and proliferative phases, body weight gain and thymus weight in the cotton pellet-induced granuloma formation[25].

T. orientalis semen extract shows anti-inflammatory activities that inhibit inflammation-associated gene expression including iNOS, COX-2, and IL-1β by blocking JNK/p38 MAPK and NF-κB pathways in lipopolysaccharide (LPS)-stimulated BV-2 mouse microglia[26]. Myristicin, an active aromatic compound found in the seed of *M. fragrans*, also showed anti-inflammatory properties by inhibition of nitric oxides, cytokines, chemokines, and growth factors in dsRNA-stimulated macrophages via the calcium pathway[27]. Ginger (*Z. officinale*), which has long been used in traditional medicine as a cure for some diseases including inflammatory diseases, was reported to significantly reduce the elevated expression of NF-κB and TNF-α in rats with liver cancer[28].

The current study shows that the RSDM formula, a combination of many anti-inflammatory plants as above mentioned, has potent anti-inflammatory activity. Not only inhibit the pro-inflammatory mediators such as nitric oxides, cytokines, chemokines production from the immune cells, but also suppress the up-regulation of inflammation associated gene expression including iNOS, COX-2, and NF-κB pathways. Thus, the beneficial effects of the RSDM formula in patients with hemorrhoids may be attributed to its anti-inflammatory activities. This supports the traditional use of the RSDM formula as a potential therapeutic agent for the treatment of inflammatory related diseases, especially the one that affected the blood vessel wall and conjunctive tissue.

There is evidence that oxidative stress has linked to the cause of inflammation. Therefore, antioxidant activity of the component plant in this formula might be beneficial in order to reduce inflammation at the site. Besides, the antioxidant effects of some component plants of the formula might contribute to the anti-inflammatory effects of the whole formula. In order to provide a point of reference, more than one antioxidant methods were applied to measure the antioxidant activity and total phenolic contents of the whole formula and 22 individual component plants extracts.

The DPPH radical scavenging assay is the most commonly employed to determine anti-oxidant activity. This method was successfully used in this study to systematically assess the total antioxidant capacity of the medicinal herbal extracts, being a simple, fast, reliable and inexpensive technique, and also very adaptable to both hydrophilic and lipophilic systems. This effective and efficient method can be used for screening of medicinal plants for their relative antioxidant content[29]. The DPPH radical scavenging activity of the component plants extracts ranged from IC_{50} = 4.4 to > 200 µg/ml. The ethanolic extract of the RSDM formula exhibited lower DPPH free radical scavenging activity than both trolox and rutin, while some of the component plants extracts, including *T. chebula*, *C. bejolghota* and *C. verum*, showed greater activity.

Many studies have found that there is a direct relationship between antioxidant

activity and total phenolic content. Phenolics are a class of plant secondary metabolites that contain one or more hydroxyl (-OH) groups attached to a benzene ring or other complex aromatic ring structures. Their radical scavenging ability is due to these hydroxyl groups. The extracts of *T. chebula*, *C. bejolghota* and *C. verum* had higher total phenolic contents than the other component plants, while the plant containing the lowest phenolic content was *P. nigrum*. Statistical analysis of these samples revealed that the antioxidant activity of the tested component plants was significantly correlated with total phenolic content ($r = 0.7$). The higher total phenolic contents of individual herbs (e.g. *T. chebula*) resulted in higher total antioxidant capacity. The ethanolic extract of the RSDM formula exhibited significant inhibition of lipid peroxidation which was superior to that of trolox and rutin. Thus, the phenolic compounds in this formula might not be the only group of contributor that inhibit this reaction.

5. CONCLUSION

The RSDM formula has been found to have anti-inflammatory and antioxidant properties. In our *in vivo* experiment, the RSDM formula has almost equal or slightly superior anti-inflammatory effects, depending on dosages used, when compared with a conventional non-steroidal anti-inflammatory drug indomethacin. Also, this herbal formula can be used as hemorrhoid treatment due to its antioxidant and phenolic contents.

Overall, this study has been done in favor of the use of RSDM formula in mild to moderate hemorrhoids. However, this might be ineffective in higher degree of hemorrhoid disease which needs more aggressive treatment under medical supervision. More *in vivo* models and clinical trials comparing with conventional treatment for hemorrhoids are required in order to create a strong body of evidence towards definite recommendations for use. Further studies involving the purification of the active constituents and investigations into the biochemical pathways may result in the development of a potent anti-inflammatory agent with low toxicity and a better therapeutic index.

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