

## Research Article

# Prevalence of Alpha-1 Adrenergic Receptor in Haemorrhoidal Tissue

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- Hemorrhoids
- Micronized flavonoid

## Abstract

This study is a pilot study to identify the presence of Alpha-1 Adrenergic receptor in hemorrhoidal tissue and to analyze the association between the presence of Alpha-1 Adrenergic receptor and the clinical effect of taking micronised flavonoid.

**Method:** Immunofluorescence method was performed on hemorrhoidal tissue taken from patient with second to fourth degree hemorrhoid from 1 June 2012 to 30 November 2013. The primary outcome was identification of the presence of Alpha-1 Adrenergic receptor in hemorrhoidal tissue and secondary outcome to clinical improvement of symptoms with usage of micronized flavonoid.

**Result:** Total number of 34 patients was recruited. There were 18(52.9%) Malay, 14(41.2%) Chinese and 2(5.9%) others. 15(44.1%) were male and 19(55.9%) were female patients. 4(11.8%) were second degree disease, 14(14.2%) were third degree disease and 16(47.1%) were fourth degree disease. 15(44.1%) did not take micronized flavonoid within 6 months prior to inclusion and 19(55.9%) took micronized flavonoid before. Immunofluorescence study showed 26(76.5%) samples contained Alpha-1 Adrenergic receptor and 8(23.5%) did not. Two (5.9%) of the receptors produced signal strength at 1+, 12(35.3%) at 2+, another 12(35.5%) at 3+. Of those patients who used micronised flavonoid within 6 months of surgery, there was no correlation between presence of Alpha-1 Adrenergic receptors and subjective clinical improvement of symptoms (Pearson Chi-Square Test = 0.656).

**Conclusion:** Alpha-1 Adrenergic Receptor is detected in hemorrhoidal tissue in majority of the patients. However there was no correlation between presence of the receptor and subjective clinical improvement of symptoms with usage of micronized flavonoid. This may suggest that micronized flavonoid does help in relieving symptoms even in absence of the receptors. A larger sample in the future would be useful to see the quantitative analysis of clinical improvement with micronised flavonoid in the presence of Alpha-1 Adrenergic receptors.

## ABBREVIATIONS

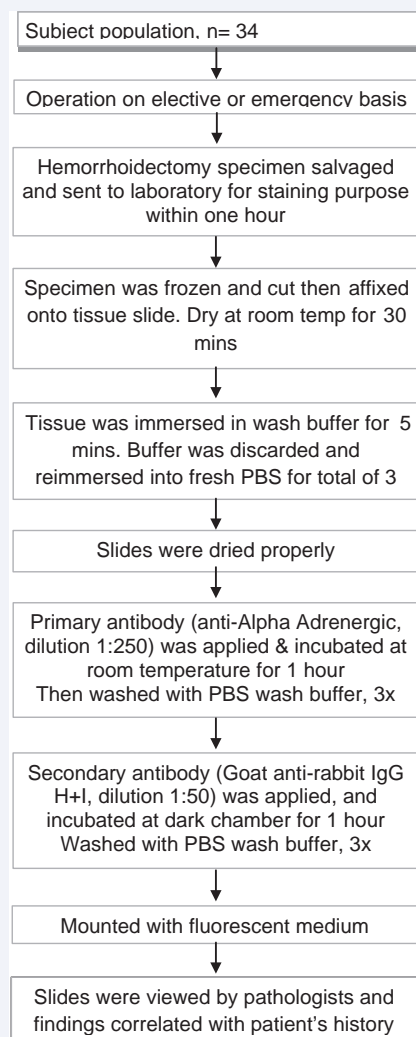
RM: Malaysian Ringgit; IF: Immunofluorescence

## INTRODUCTION

Hemorrhoid is an anorectal disorder associated with bleeding and prolapsed of the anal cushion, which is a highly vascularized tissue comprising of arteriovenous anastomosis [1,2]. The anal cushions are thought to make a significant contribution to the mean anal resting pressure and the maintenance of continence [3]. They contribute up to 15-20% of the resting anal pressure, by intensifying the action of the anal sphincter mechanism and shield the anal canal and the anal sphincter during the act of

evacuation by filling with blood and providing extra padding [4]. Many patients experience this condition without seeking medical consultation due to embarrassment or fear. Studies have shown that about 10 million of United States citizens for a prevalence of 4.4% and 13-36% of United Kingdom populations have reported hemorrhoids [1]. In both gender, a peak in the prevalence is noted between 45 to 65 years old [4,5].

Hemorrhoids can be classified according to their origin. The dentate line serves as an anatomical histology border [5]. External hemorrhoids originate distal to the dentate line, arising from the inferior hemorrhoidal plexus, are lined with the modified squamous epithelium, which is richly innervated with



Flow Chart

somatic pain receptors (delta type, unmyelinated ) [5,6]. Internal hemorrhoids originate proximal to the dentate line, arising from the superior hemorrhoidal plexus, and are covered with mucosa layer only [4,16]. Internal hemorrhoids are further classified into four grades according to the extent of prolapsed. In the first degree, the hemorrhoidal tissue protrudes into the lumen but not prolapsed outside the anal canal and may bleed during evacuation of feces. Second degree may prolapse but spontaneously reduced post evacuation. Third degree hemorrhoids protrude outside and required manual reduction and fourth degree are irreducible [1,4, 5]. The image of internal hemorrhoid grading as shown in Figure 7.

The treatment of hemorrhoids depends on the severity of the disease. It may be just simple advice on lifestyle modification, office procedure or even surgical intervention. Patients are normally advised to improve anal hygiene, increasing the intake of dietary fiber and fluid and avoiding constipation or diarrhea [6]. In Europe and Asia, vasotonic drugs are commonly prescribed [4,5]. Micronized flavonoid is a commonly prescribed medication for hemorrhoids. It is a vasotonic agent whose active component is micronized flavonoidic fraction that contains flavonoid

extracts of rutaceae, equivalent to 150 mg diosmin expressed as hesperidine [8]. It claims to prolong the Noradrenaline activity and causes vasoconstriction in the hemorrhoidal tissue. Theoretically, in order to have vasoconstrictive property acted on by noradrenaline, the hemorrhoidal tissue should contain alpha-1 adrenergic receptor [9,10,11,12,17,18]. Many randomized controlled trials have shown the efficacy in alleviating the symptoms and proctoscopy appearance of the hemorrhoids with micronized flavonoids [8]. Some have even claimed to reduce the bleeding post hemorrhoidectomy [8]. But the trials were very subjective and were based on the replies of patients. Effects of micronized flavonoid on the hemorrhoids could not be ascertained.

Daflon, the most well recognized micronized flavonoid, is available over-the-counter and priced at about RM 50 per packet. Our hospital is prescribing about 200-250 packets per month. This comes to a cost of RM 120,000 to RM 150,000 annually.

This then led to a question of does the hemorrhoidal tissue actually contains alpha-1 adrenergic receptors and what is the relationship between presence of alpha-1 adrenergic receptor with clinical improvement of haemorrhoidal symptoms?

Adrenergic receptors are a class of transmembrane metabotropic, G protein-coupled receptors that are targets of catecholamine, especially noradrenaline and adrenaline [13,14]. There are two groups of adrenergic receptors, alpha and beta group. The functions of adrenergic receptors are different [10,18]. The locations of the receptors are well identified in other parts of the body and correlated to their functions. Surprisingly,

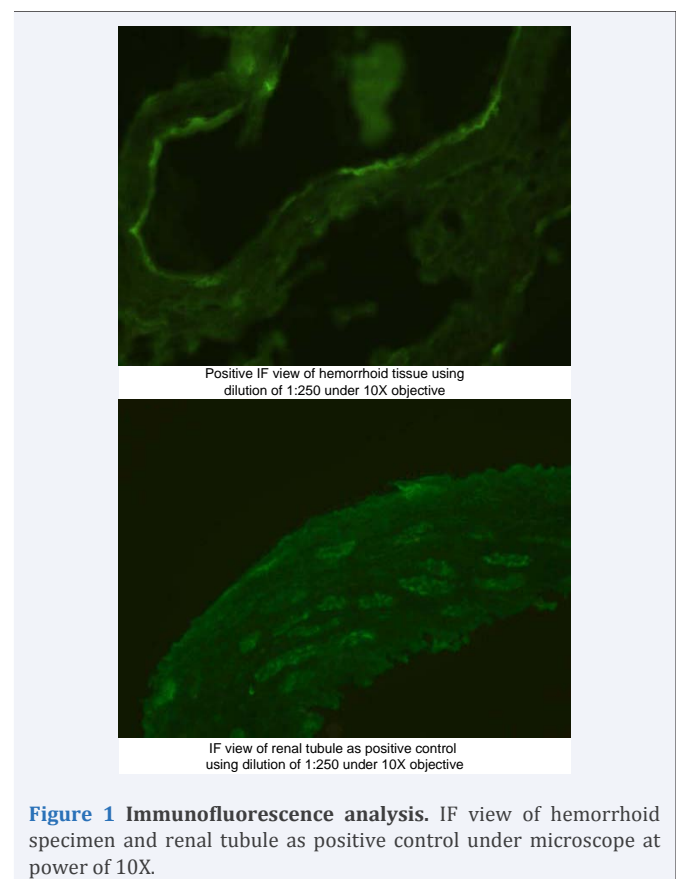
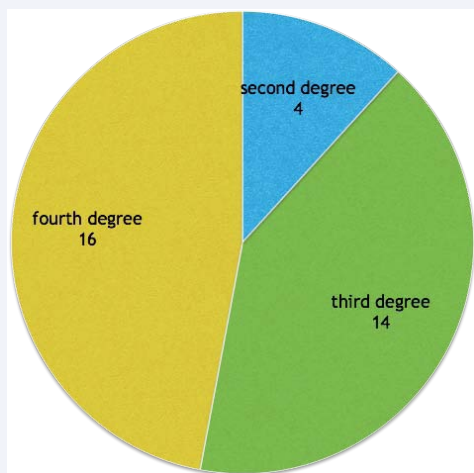
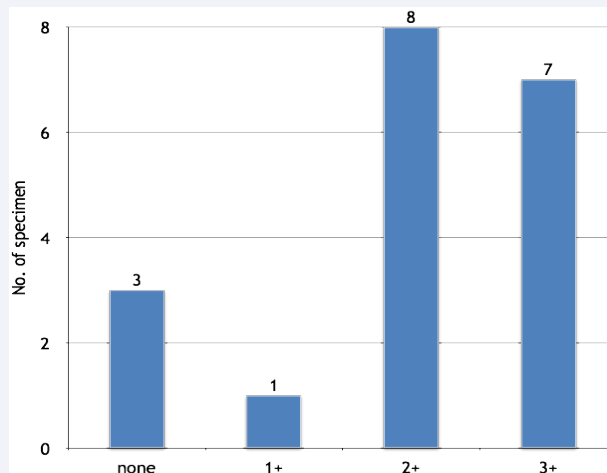


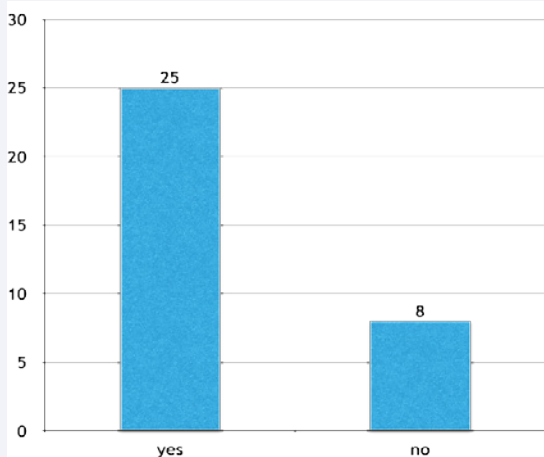
Figure 1 Immunofluorescence analysis. IF view of hemorrhoid specimen and renal tubule as positive control under microscope at power of 10X.



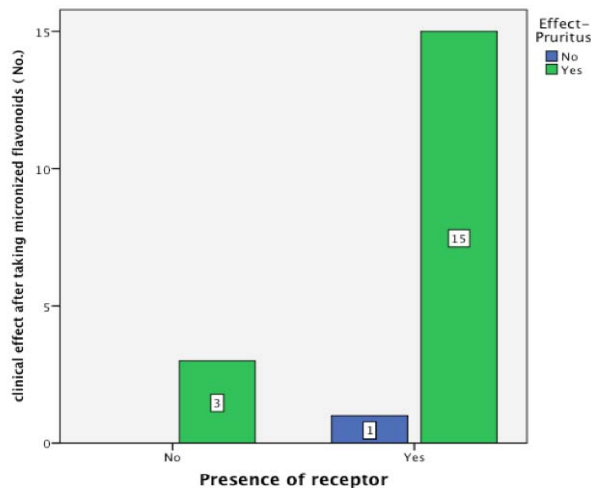
**Figure 2** Number of patient based on severity of disease.



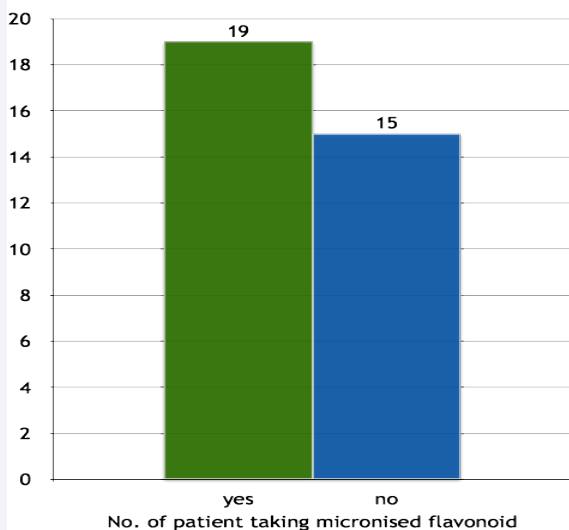
**Figure 5** Number of specimen based on signal strength of IF view in those patients taking micronized flavonoid.



**Figure 3** Number of hemorrhoidal tissue contained Alpha-1 Adrenergic receptors.



**Figure 6** No. of patient who has clinical improvement after taking micronized flavonoid with presence of receptors.



**Figure 4** No. of patient taking micronized flavonoids prior to surgery.

although hemorrhoid is known to be a common disorder, there is no major study concerning receptors present on the vasculature of the anal canal. One study has successfully identified the presence of Endothelin 1 receptors in hemorrhoidal tissue [13]. So with the randomized control trials that have recognized the effect of the micronized flavonoids [8], we want to know whether alpha-1 adrenergic receptors exist in hemorrhoidal tissue and the association between the number of receptors present and the clinical efficacy of micronized flavonoid.

**MATERIALS AND METHODS**

This study was designed as an investigational study and based solely in Hospital Sultanah Aminah Johor Bahru, Malaysia from 1 June 2012 until 30 November 2013. All patients with hemorrhoids were graded and surgical intervention was offered to those with second to fourth degree. The salvaged hemorrhoid tissue was sent to pathologists for analysis using immunofluorescence method.

Patients above 18 years old who came to Surgical Follow-up Clinic in Hospital Sultanah Aminah Johor Bahru, Malaysia diagnosed to have second to fourth degree hemorrhoids, was included into the study. They were asked regarding the medication they have been taken for the particular disease and its effectiveness.

All the patients included into the study were registered into a pre-designed performa. Data on patient's gender, ethnicity BMI, grade of disease and medication taken for the particular disease before surgery were documented.

As this is a pilot study, no reference can be made. Based on the rule of thumb, a number of 34 patients were targeted due to financial constraint. The antibodies that were used for identification of alpha-1 adrenergic receptor were bought from Lab vision. A grant was given by Universiti Kebangsaan Malaysia for the study.

The patients' hemorrhoidal disease was graded carefully. Those patients who were diagnosed with second to fourth degree disease were offered surgical intervention. Patients who consented for surgery were counseled for recruitment into the study. They were interviewed and asked regarding usage of micronized flavonoid and its effectiveness such as reduced bleeding, pain, discharge and pruritus.

The hemorrhoid tissue was salvaged during hemorrhoidectomy and sent to the pathology lab within one hour for immunofluorescence analysis. The tissues were received by a designated laboratory technician for staining purposes in order to run the test.

Test was performed on thin (5 µm) cryostats section of fresh unfixated specimen in temperature below freezing point -21 to -25 °C. All the slides were labeled by the registration number. The cryostat sections on the frosted ring slides then were dried in a stream of warm air for 30 minutes. Immunofluorescence tests were performed together with a positive control.

The cut sections were washed using phosphate buffer saline (PBS) with 3 changes in 30 minutes. The slides were dried properly and applied with antibody of alpha-1 adrenergic receptor in dilution of 1:250. They were incubated for another 30 minutes at room temperature.

After that, another washing using PBS with 3 changes in 30 minutes were done. Secondary antibody (Goat Anti Rabbit IgG H+I, dilution 1:50) was applied to all the slides and incubated again at dark chamber for one hour. Final 3 washing with PBS were done before the slides were ready to be read on fluorescent microscope by two pathologists.

The presence of the receptor was confirmed and strength of the signal of the receptors was graded based of the objective power of the microscopy (microscope model Olympus BX-41) used. Association of receptor presence and effect of micronized flavonoid was correlated using SPSS version 21.

## RESULTS

Total number of 34 hemorrhoidal tissue samples with different severity (Figure 2) were collected and sent for immunofluorescence study. Twenty-five (76.5%) contained alpha-1 adrenergic receptor in the endothelium and 8(23.5%)

(Figure 3) did not contain receptor. None of the cases showed alpha-1 adrenergic receptor in the smooth muscles of the blood vessel (Figure 1).

Out of the 34 patients who were recruited into the study, 19(55.9%) patients took micronized flavonoid within 6 months prior to the surgery (Figure 4). Sixteen (84.2%) of the 19 hemorrhoidal tissues were noted to contain Alpha-1 Adrenergic receptor whose immunofluorescence signal ranging from 1+ to 3+ (Figure 5). However 18 out of 19 patients who were analyzed, claimed to have relief of symptom after taking micronized flavonoid (Figure 6).

The association of the clinical effect after taking micronized flavonoids and presence of Alpha-1 Adrenergic receptors was analyzed using Pearson Chi-Square test.  $p=0.656$  which show no significant relationship.

## DISCUSSION

Hemorrhoid is one of the most common anorectal disorder treated in our surgical clinic. Surgical intervention is commonly offered to those presenting with fourth degree hemorrhoid [4,5,6,15]. However, patients tend to favor non-invasive procedure such as medical treatment. Micronised flavonoid is the most common drug that is used to treat hemorrhoid [6,8]. Currently, there was no study that convincingly confirmed the effectiveness of the drug.

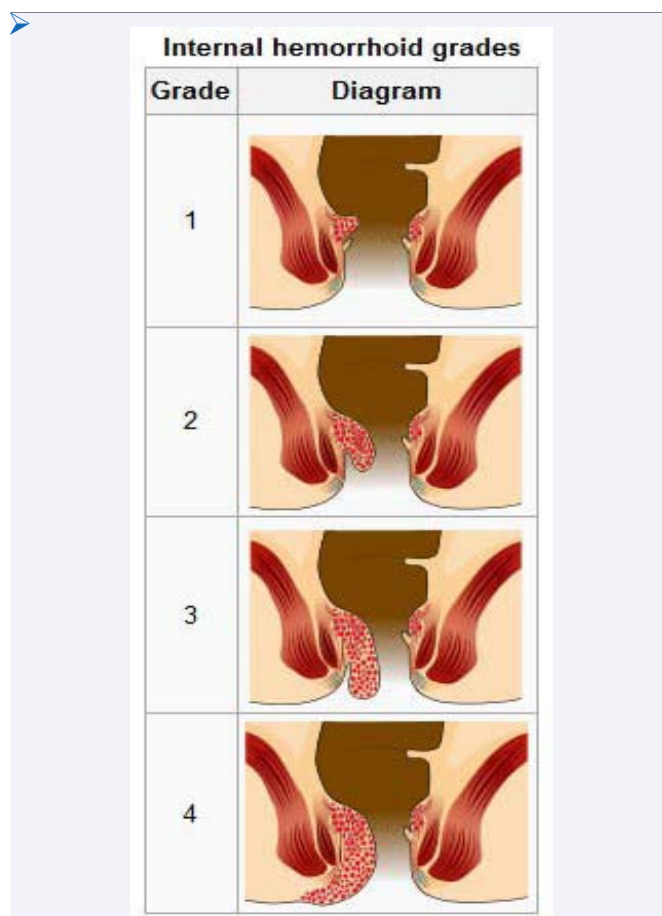


Figure 7 Internal Hemorrhoid Grading [Image on the Internet] [7].

Micronised flavonoid is used as a vasoconstrictive agent for hemorrhoid [8]. We speculated activation of the receptors in endothelium to release the adenosine triphosphate (ADP), which causes contraction of the smooth muscles, leading to vasoconstriction [10]. This study was mainly to study the presence of the alpha-1 adrenergic receptor within the hemorrhoidal tissues.

In our study, 34 samples of hemorrhoid tissues were sent for analysis. As far to our knowledge there was no previous study, which successfully excised the fragile hemorrhoid tissue for analysis for alpha-1 adrenergic receptors. We managed to excise and stained the hemorrhoid tissue with the collaboration of the pathology laboratory personnel.

Analysis was done under recommended protocol and strictly follows per protocol. Pathologist read the specimen slide after agreeing the signal grading reference in view of no reference prior to this. Signal strength was graded based on the objective power of microscope used.

Result showed only 19 (55.9%) patients were taking micronised flavonoid prior to the surgery and 15 (44.1%) patients didn't. So, the analysis was performed on these 19 patients. Of those 19 patients, 16 patients were noted to contain Alpha-1 adrenergic receptors. This means that, not all patients with hemorrhoids have the receptors within their hemorrhoidal tissues, which is supposed to serve as the theoretical vasoconstrictive mechanism for micronized flavonoids.

To make the findings more interesting, 3 patients who have no alpha-1 adrenergic receptors also claimed to have symptoms relieved with micronised flavonoids. This has led to a new thought that suggests a different mechanism of micronised flavonoid to relieve the symptoms. A larger sample size and better design of protocol may be required to account for probability of comorbidities that reduces the sensitivity of the receptor in order to analyze the relationship of micronised flavonoid and alpha-1 adrenergic receptors.

## CONCLUSION

The study showed that the majority of hemorrhoidal tissue does contain Alpha-1 Adrenergic receptor. However, there is no statistically significant correlation of clinical efficacy of micronized flavonoids and the presence of Alpha-1 Adrenergic receptors in hemorrhoidal tissue. This may suggest that micronized flavonoid acts via another mechanism and it does have a role in relieving the symptoms of hemorrhoid.

The pathway of action by micronized flavonoid to relieve the hemorrhoid symptom is still unclear and further study is warranted to explore the actual mechanism of micronized flavonoid.

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