LEMBAR
HASIL PENILAIAN SEJAWAT SEBIDANG ATAU *PEER REVIEW*
KARYA ILMIAH : JURNAL ILMIAH

Judul Karya Ilmiah : Phlebotrophic Effect of Graptophyllum Pictum (L.) Griff on Experimental Wistar Hemorrhoids
Jumlah Penulis : 7 Orang
Status Pengusul : Mario SadarBernithoHutagalung, Bernadus Parish Budiono,Sigit Adi Prasetyo, Ignatius Riwanto, Eriawan Agung Nugroho,**Yan Wisnu Prajoko**, Neni Susilaningsih
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Letters to the Editor

Seroprevalence of Hepatitis B and C in Healthy Malaysian Adults: A Preliminary Report
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Hui-min Neoh, Saberi bin Saimun, Rahman Jamal

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JOURNAL OF BIOMEDICINE AND TRANSLATIONAL RESEARCH

Letter to The Editor

Seroprevalence of Hepatitis B and C in Healthy Malaysian Adults: A Preliminary Report

Hui-min Neoh, Saberi bin Saimun, Rahman Jamal*

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Viral hepatitis caused by Hepatitis A, B or C virus infection is a significant cause of morbidity and mortality in Malaysia. The national incidence rate for hepatitis A has dropped steadily in conjunction with successful public health measures; nevertheless, hepatitis B and C incidence and its clinical outcome remain a concern for health authorities[1].

In this study, previous hepatitis B (HBV) and C (HCV) virus prevalence in healthy Malaysian adults residing in Peninsular Malaysia was determined in a serosurvey. One thousand and twenty (1020) serum samples were randomly sampled from the Malaysian Cohort biobank. The Malaysian Cohort is a national project carried out to determine risk factors and biomarkers for diseases in the Malaysian population[2] . For this serosurvey, samples were from participants aged 35-64 and residing in the states of Peninsular Malaysia. HBV and HCV prevalence of sampled subjects were determined using HBV surface antigen (HBsAg) and HCV antibody (HCV Ab) detection kits (RVR Diagnostics Sdn. Bhd., Malaysia), respectively. Briefly, 10ul of each tested serum was pipetted into the well of a test cartridge, which was later added with the kit's diluent reagent. The sample and diluent mixture was then incubated for 10 minutes prior reading of test result. A double band on the cartridge (one band for control, another for sample positivity) indicates a positive result, while a single band is indicative of a negative outcome. The HBsAg kit has a sensitivity and specificity of 100%, while the HCV Ab kit has a sensitivity of 98.7% and specificity of 99.6%.

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Twenty-eight (28) or 2.8% serum samples were tested positive for HBsAg, while 10 (1.0%) samples were found to carry antibodies for HCV. No sample was positive for both HBsAG and HCV Ab. Interestingly, for the HBsAg positive samples, most (n=14, 50.0%) were from the older age group of 55-64 years old, and staying in rural areas (n= 18, 64.3%). While for the HCV seropositive subjects, most were from the older age group of 55-64 years old (n= 5, 50%), with more urbanites found to be seropositive (n= 6, 60%).

The prevalence of HBsAg in Malaysians was reported to be around 5 to 7% in the 1980s; this was before the nationwide implementation of hepatitis B vaccination for all new-born infants in 1989[3]. Even though the vaccination drive was aimed at infants, some young adults during the 1980s also received the vaccination; this might have subsequently lowered the seroprevalence to 2.8% as found in this study[4]. However, a previous report estimated 2.5% of the Malaysian population aged 15-64 years old to be positive for HCV exposure, an estimate higher than the results obtained in this study[5]. Despite its low prevalence, HCV seropositivity remains a healthcare concern as no vaccine is yet available; and without active screening surveillance the infected are usually diagnosed at a later stage with cirrhosis or liver cancer. In Indonesia, to our knowledge, only one study on HCV seroprevalence was perform in healthy subjects, where 2.2% were found to be HCV positive[6]; while HBsAg prevalence was found to be 1.1% in young adults of Singapore[7].

This preliminary report shows low seroprevalence of HBV and HCV in adults residing in Peninsular Malaysia. A larger study involving representative participants for each demographic factor will be carried out in the future.

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Review Article

Fragile X syndrome, the search for a targeted treatment

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Abstract

Background : Fragile X syndrome (FXS), the most common monogenetic cause of intellectual disability and autism spectrum disorders, is characterized by behavioral and physical problems. There is currently no adequate treatment available. While animal model studies seemed extremely promising, no success has been achieved in the larger clinical trials with human FXS patients. This short review describes the steps that have been taken in the development of a targeted treatment for FXS. Possible reasons for the lack of translation between animal models and human FXS patients are being explored and solutions are being proposed. The FXS story illustrates pitfalls and possibilities in translational research, that might especially be applicable for other neurodevelopmental disorders as well

Keywords FMR1, fragile X syndrome, Fmr1 KO mouse, mGluR5, GABA, clinical trial, outcome measure
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INTRODUCTION

Fragile X syndrome (FXS) is the most common monogenetic cause of intellectual disability and autism spectrum disorders, affecting about 1:7000 males [1,2]. The disorder is caused by a CGG repeat expansion in the 5' UTR of the *FMR1* gene. This repeat expansion leads to silencing of the *FMR1* gene and lack of its protein product, FMRP. Since FMRP plays an important role in regulation of synaptic plasticity in the brain, its lack leads to several neurocognitive and behavioral problems. Hence, FXS is accompanied by intellectual disability, autism spectrum disorders, executive function deficits, attention and hyperactivity disorder, aggression and anxiety, amongst others. Also medical problems are frequent, including epilepsy and frequent otitis media in children [3,4]. Patients are usually attending special education or end up in institutions. Emotional and behavioral problems are most disabling and are often treated with non-specific symptomatic pharmacological and supportive treatments. However, these interventions are mostly insufficient and there is no disease-modifying effective therapy, targeting the cognitive, behavioral, emotional or medical problems.

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This lack of effective therapy leaves parents, care-givers and medical professionals with little options to alleviate the burden of taking care of a patient with FXS. Moreover, its relatively high frequency together with the life-long intellectual, and sometimes extreme behavioral and physical disabilities and the hereditary character of the disease, make FXS very costly for society. Hence, an effective targeted disease modifying therapy, especially affecting the behavioral, emotional and intellectual problems, is important for patients, caregivers, physicians and society.

THE SEARCH FOR A TARGETED TREATMENT

Identification of the *FMR1* gene as the causative gene [5], opened possibilities to study the disease in animal models. In the past decades, much research has been performed on FXS animal models, for example the *Fmr1* knock-out (KO) mouse, the fruit fly and the zebrafish. This research identified the function of the *FMR1* protein product, FMRP, as a key regulator of the neuronal synaptic plasticity, by binding and regulating the local translation of target mRNAs. Many synaptic pathways have been shown to be disturbed in FXS [6]. A few of these include the metabotropic glutamate type 5 receptor (mGluR5) pathway, the gamma-Aminobutyric acid (GABA)ergic pathway, the endocannabinoid pathway, the matrix metalloproteinase 9 (MMP9) pathway and intracellular signaling pathways (e.g. extracellular signal related kinase (ERK), mammalian target of rapamycin

Phlebotrophic Effect of Graptophyllum Pictum (L.) Griff on Experimental Wistar Hemorrhoids

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Research Article

Phlebotrophic Effect of *Graptophyllum pictum* (L.) Griff. on Experimental Wistar Hemorrhoids

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Abstract

Background : *Graptophyllum pictum* extract (GPE) has already been used widely in Indonesia to treat hemorrhoid with good result, however, the mechanism is not supported by the molecular research. GPE has the potential effect as an anti-hemorrhoidal drug through the phlebotropic mechanism.

Objective : To study the phlebotropic effects of GPE by measuring the degree of edema and extra vassal leucocytes of experimental Wistar hemorrhoid.

Methods : An experimental study in male Wistar rats, weight around 200 gr, induced for the development of a disease-like condition of hemorrhoids by 6% croton oil induction on the anus for 3 days. Fourteen Wistar rat were randomly allocated into 2 groups. Group I got normal saline, group II was treated with GPE 100mg/kgbw, started on day 4th for 5 consecutive days. On 9th day blood was extracted from retroorbital fossa and anus was resected up to 2 cm from anal verge and weighted. The degree of anal edema was measured by recto anal coefficient and the number of extra vassal leucocytes was measured from HE staining of anal specimen under 400 HPF. All of the data showed normal distribution, therefore, pool t-test was used to test the mean difference between groups.

Results : The mean (±SD) of recto anal coefficient in the treatment group was 2.46 (±0.41) and it was significantly lower than control group (3.13 ± 0.85) (p = 0.029). The mean (±SD) of extra vassal leukocytes in the treatment group was 900.14 (±48.09) and it was significantly lower than the control (1003.28 ± 99.30) (p = 0.042).

Conclusions : *Graptophyllum pictum* extract shows a phlebotropic effect in terms of decreased recto anal coefficient (edema) and decreased of extra vassal leukocytes in Wistar rats.

Keywords : *Graptophyllum pictum*; hemorrhoids; croton oil; recto anal coefficient; extra vasal leucocytes
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INTRODUCTION

The number of patients diagnosed with hemorrhoids is increasing annually. Treatment options are based on their pathological degree. First, 2nd and small 3rd degree of hemorrhoid can be managed non-operatively.[4] Medical treatment given is a drug that has the effect of being anti-inflammatory and phlebotropic.[2,5]

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Micronized purified flavonoid fraction (MPFF) has been known to reduce the symptoms of bleeding, pain, and recurrence of hemorrhoids,[11,12] the effectiveness has already been proved on meta-analysis of RCT study.[8] However, this drug is not included yet in the Indonesian national formulary, therefore it is not allowed to be given to patients covered by national insurance.

Graptophyllum pictum (L.) Griff. or purple leaves extract has been widely used in Indonesia as an alternative medicine to treat several kinds of diseases,

including hemorrhoid, is potential to be developed as an alternative hemorrhoid therapy. Purple leaves contain alkaloids, flavonoids, tannins and steroids and antioxidants. The entire content above will be produced well by extracting with 70% ethanol.[6] Review of various studies conducted by Singh et al., showed that purple leaves contain alkaloids, glycoside, pectin, formic acid, steroids, saponins, tannins, flavonoids and alcohol.[10] Purple leaves as anti-inflammatory have been proven to play a role, through intervention studies in experimental rats, and concluded that the strength is equivalent to indomethacin.[7,8,9] An experimental study in rats which also made artificial hemorrhoids by anal induction with 6% croton oil, but treated with topical cream a combination of several herbal extracts showed anti-inflammatory and antioxidant effects compared to control.[10] Referring to previous studies, purple leaf has the potential to be developed as an anti-hemorrhoidal drug as anti-inflammatory and phlebotropic. The previous study on Wistar rat use *Graptophyllum pictum* (L.) Griff. extract at dose 100 mg, 150 mg and 200 mg/kg body weight, and at dose 100 mg/kg body weight had already shown to reduce the blood level of TNF-alfa and IL-6 significantly,[10] therefore this study used dose 100 mg/kg body weight. This study is expected to show the role of purple leaves even deeper as phlebotropic in terms of reducing vascular leak by measuring number of leukocyte extra-vassal and degree of anal edema.

MATERIALS AND METHODS

Subject

This study was an animal experimental research model. The animal were healthy male adult Wistar rats at the age of 10-12 weeks, with the weight around 200 g. The Wistar were obtained from the animal house unit of the Lembaga Pengembangan Penelitian Terapan (LPPT) University of Gajahmada, Jogjakarta, and the experiment was also done in LPPT. The animal were excluded if during 7 days observation appeared to be sick or death. All rats came from the same strain, and received the same treatment during the trial period. Both the control group and the treatment group were given the same amount of food and drink and were placed in 2 different cages. Guide for the care and use of Laboratory Animals were applied completely to all Wistar rats under experiment.[15] We used "resource equation" method to calculate the sample size.[16] According to this method a value "E" is the degree of freedom of analysis of variance. The value of E should lie between 10 and 20. E can be measured by following formula: E = Total number of animals − Total number of groups. In our study the total number of animals were 14, and total number groups were 2, mean E was 12, it is meet the requirement. This study has obtained Ethical Clearance from "Komisi Etik Penelitian Kesehatan, Fakultas Kedokteran Universitas Diponegoro dan RSUP dr Kariadi Semarang" no 72/EC/H/FK-RSDK/IX/2017.

Croton oil

The croton oil was provided from Sigma Aldrich company, catalog number C6719-10G. Croton oil for anal application was prepared as combine mixture of Deionized water, pyridine, diethyl ether, and 6% croton oil in diethyl ether at a ratio of 1: 4: 5: 10. The night before, all of the Wistar were refrained from foods, and then with sterile cotton, 6% croton oil were put into the anus at 1.5 cm deep and maintained for 30 seconds, in 3 consecutive days[15].

Graptophyllum pictum (L.) Griff. extract

Graptophyllum pictum (GP) is member of Acanthaceae family or Justicia picta, is believe to be native of New Guinea,[10] but nowadays it can be found in tropical country including Indonesia. GP leaves were harvested from the Sido Muncul herbal medicine factory farm, in Semarang, Indonesia. The extraction processes were also done in this factory. GP powder was extracted with 70% ethanol using soxhlet extractor, which was then concentrated in a vacuum container to achieve 95% concentration, and stored at 15-20° C.[18, 19] The dose of GPE was 100 mg/kg given twice daily intravenous, as already been used by the previous research. [17,20]

Experimental design

On the day 4, the day after finishing anal induction with 6% croton oil, the Wistar rats were randomly allocated into 2 groups. Group I (control), starting from the 4th day was given physiological saline for 5 consecutive days. Group II (treatment group), on the 4th days was given GPE at a dose of 100 mg/kgbw for 5 consecutive days. GPE was given intravenously.

During the treatment period, all animals were cage based on group and feed accordingly with plenty water. On the 9th day after induction, all rats were terminated by cervical dislocation under ether anesthesia. The Wistar rat was weighted by using gram scales. The anus was resected up to 2 cm above anal verge, and weight by using milligram scales. The anal specimen was saved in the formalin buffer container, and preparing for microscopic examination by HE staining under 400 HPF.

To evaluate the edema, because the walls are very thin, it is not accurate if the anal wall is measured with a millimeter ruler. It was believed that edema will increase the anal weight, and anal weight will also dependent to the Wistar weight. Therefore, recto anal coefficient would be more reliable. Based on previous study, the degree of anal edema could be measured using recto-anal coefficient, that is ratio between anal weight (in miligram) to Wistar body weight (in gram). [17] This study examined extra vasal leukocyte counts and recto anal coefficient.

Statistical analysis

Both variables were normally distributed based on Kolmogorov-Smirnov test. Pool t test was used to test the differences on extra vasal leukocyte counts and recto anal coefficient between control and treatment groups.

RESULTS

All Wistar rats were still in good health until the end of the study. At the end of the experiment, we measured body weight using gram scales, where the control group was 173.84 ± 13.37 and the treatment group was 171.70 ± 13.10, and statistically it was no significant different between the two groups (p = 0.833). Figure 1 showed that recto anal coefficients in the group of GPE was 2.46 ± 0.41, and was significantly lower than the control group 3.13 ± 0.85 (p = 0.029). Figure 2 showed that number of

extra vassal leukocyte in the group GPE was 900.14 ± 48.09 and was significantly lower than the control group 1003.28 ± 99.30 (p= 0.042).

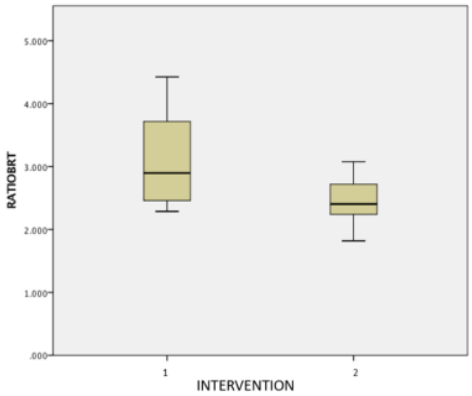

Figure 1. Recto anal coefficients in the GPE group (2) was 2.46 ± 0.41 and control group (1) was 3.13 ± 0.85, (pool t test p = 0.029).

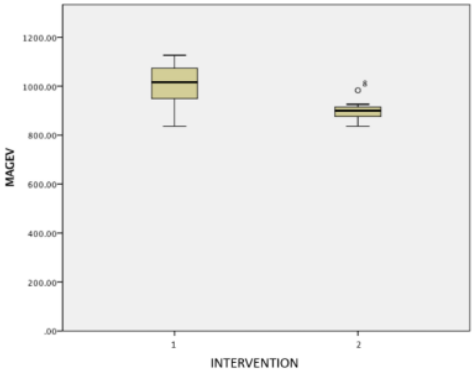

Figure 2. Number of extra vasal leukocytes in the GPE group (2) was 900.14 ± 48.09, and the control group (1) was 1003.28 ± 99.30, (pool t test p = 0.042)

DISCUSSION

This experimental research can be carried out well, because 14 male Wistar rats aged 2-3 months, which were randomized into 2 groups (each of 7 tails), could survive all at the end of the study and all seemed healthy and active. This research is to see the phlebotropic effect of purple leaf extract. Second hemorrhoid degree arised as a result of the induction of 6% croton oil, and inflammation in the anus occured, where one component is edema due to vascular leakage, so that the anal wall would be thicker and heavier[15]. Acute inflammation resembled to acute hemorrhoid. Using 6% croton oil to induce hemorrhoid was in accordance with previous research.[21, 22] Irritation by croton oil may damage mucous cell that will release alarmin or danger associated molecular patterns (DAMPs). DAMPs has capacity to induce innate and adaptive immunity by activating inflammation-related pathways. The proinflammatory interleukin induce vasodilatation and vascular leak.[23]

In this study, purple leaves has a significantly lower recto anal coefficient than the control group, which was indicated by reduced edema after administration of purple leaf extract 100 mg / kg body weight. Because edema occurs due to vascular leakage, so it can be said, purple leaves have a phlebotropic effect. The presence of leukocyte extravasation is also an indicator of a state of vascular leakage and inflammation[3]. In this study, the number of extra vasal leukocytes was significantly lower in the group of purple leaf extracts compared to the control group. This can be concluded that administration of 100 mg/kgbw of purple leaf extract can reduce leukocyte extravasation, and purple leaf has plebophtopic effect. It was in accordance with the research of Ozaki et al and Sari et al.[11,12]

Mechanism of decreasing edema and extravasal leucocyte by purple leaf extract is not known yet. The active component of purple leaft extract is flavonoid. MPFF significantly reduced the extent of pain and bleeding in the selected subjects of this study with acute haemorrhoids. The active component of MPFF is also flavanoid, therefore it can be suspected that the mechanism action of purple leaft extract may be resemble with MPFF. From the review of previous study, MPFF inhibits endothelial activation and prevents inflammatory cascade resulting from leukocyte-endothelium interaction.[24] Curcumin from curcoma longa, sulforaphane and iberin from cruciferous vegetables have anti inflammatory effect through their antagonist activity of Toll-Like Receptor 4.([24,25]) Study to know whether MPFF has Toll-Like Receptor 4 antagonist activity should be done.

CONCLUSION

Purple leaf extract shows a phlebotropic effect on artificial hemorrhoids of Wistar rats by decreasing recto anal coefficient and decreasing extra vassal leukocytes. Further study to elaborate the role of purple leaves extract on inhibiting endothelial activation or has Toll-Like Receptor 4 antagonist activity is proposed.

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