Pharmacogn J. 2020; 12(6)Suppl: 1489-1493
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original Article

Evaluation of *Myrmecodia pendans* Water Extracts on Hematology Profiles, Liver, Kidney Function and Malondialdehyde Level in Healthy Volunteer

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ABSTRACT

Background: Ant Nest (*Myrmecodia pendans*) is one of plants that have been used by locals in Indonesia to empirically treat various diseases. Ant Nest *in vitro* and *in vivo* studies on animals have been widely reported its pharmacological activities as an antioxidant. Unfortunately, scientific proofs reported on this plant as human medicine are still lacking. **Aim:** This study aimed to ensure effectiveness, and safety due to administration of Ant Nest Water Extract (ANWE) formulated in capsule. **Materials and Methods:** Twelve volunteers were divided into 2 groups, 6 volunteers in each group. Group I: Ant Nest Water Extract Capsule (ANWEC) in dose of 350 mg ANWE, and group II: Placebo Capsule (PC) which contained 350 mg amylum. Each groups given the capsule once daily for 28 days. The comparison was made between group I and group II. Hematology tests include hemoglobin, leucocyte and thrombocyte. Blood chemistry tests include SGOT, SGPT, total cholesterol, triglycerides, HDL, LDL, glucose, ureum (Ur) and creatinine (Cr). Malondialdehyde (MDA) level was measured at day-28 which was the last day of the intervention. **Results:** After 28 days administration of ANWE, the SGOT, SGPT, Ur, and Cr showed in normal level as follows SGOT (U/L): 19.2 ± 3.99; SGPT (U/L): 17.2 ± 6.80; Ur (mg/dL): 19.75 ± 3.66; Cr (mg/dL): 1.06 ± 0.13. MDA (ng/mL) level in ANWEC-treated group was significantly lower (117.2±23.8) than PC (147.25±18.7). There was no intolerable complaints during the observation. **Conclusion:** The study concluded that Water Extract of Ant Nest 350 mg has no damage to liver, kidney and hematology, so it was proven that this plant is safed to be consumed by human for its potency as antioxidant.

Key words: Antioxidant, *Myrmecodia pendans,* Malondialdehyde, Safety.

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History

- Submission Date: 23-06-2020;
- Review completed: 04-08-2020;
- Accepted Date: 10-08-2020.

DOI : 10.5530/pj.2020.12.204

Article Available online

http://www.phcogj.com/v12/i6s

INTRODUCTION

Ant Nest (*Myrmecodia pendans*) is one of plants that have been used by locals in Indonesia to empirically treat various diseases, starting from mild diseases to severe diseases such as nausea, breast cancer,[1] leukemia, heart diseases, tuberculosis, kidney and prostate dysfunction, various allergies, migraine, rheumatism, and hemorrhoid.[2] It is also known to have antioxidant, antimicrobial and immunomodulatory activities.[1] *M. pendans* belongs to the Rubiaceae family. It is typically found in Indonesia, particularly in Papua and areas with tropical forests such as Sumatra, Borneo, and Toraja.[3] Generally, the part used as medicine is hypocotyl (tubers) by drinking the boiled tissue in water (decoctum).[4]

The theory of an illness process is associated with the acivity of free radicals. Free radicals are atoms or groups with unpaired electrons, and they are generally unstable and highly reactive.[5] When an overload of free radicals cannot gradually be destroyed, their accumulation in the body generates a phenomenon called oxidative stress. This process plays a major part in the development of chronic and degenerative illness such as cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases.[6,7]

Malondialdehyde (MDA) increases in body during excessive oxidative stress.[8] The hypothesis of oxidative stress highlights the crucial role of antioxidant defenses as an important component of the overall balance of the organism.[9] MDA estimations can be used as a reliable tool to assess oxidative stress levels.[10]

The term of antioxidant refers to a compound that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions and which can thus prevent or repair damage done to the body's cells by oxygen.[11,12] Natural antioxidants widely reported in medicinal plants, including Ant Nest (*M. pendans*. Ant Nest is one of the natural sources that can be used as an alternative medicine associated with its activity as an antioxidant.[4] The active fraction contained saponin, alkaloid, tannin and flavonoids.[13] This plant is potential to be developed in modern herbal medicines because it can grow well as ephyphyte, therefore the exploitation will not endanger the environment.[1]

Some herbal therapies may cause potential toxicity.[14] Therefore, in order to be used widely like conventional medicine, medicinal plants must be supported by studies and evidence for their efficacy and safety on body systems, especially blood, liver and kidney function.

Cite this article: Widyawati T, Pase MA, Daulay M, Sumantri IB, Yusoff NA. Evaluation of *Myrmecodia pendans* Water Extracts on Hematology Profiles, Liver, Kidney Function and Malondialdehyde Level in Healthy Volunteer. Pharmacogn J. 2020;12(6)Suppl:1489-93.

Ant Nest *in vitro* and *in vivo* studies on animals have been widely reported its pharmacological activities as an antioxidant.[15,16,17,18] Unfortunately, scientific proofs reported on this plant as human medicine are still lacking. This study aimed to ensure effectiveness, safety and description of side effects that can arise in humans due to administration of Ant Nest Water Extract with a certain dose and dosage form.

MATERIALS AND METHODS

Plant material

M. pendans leaves were purchased from Papua Island, Indonesia. The voucher specimen was identified and deposited in the herbarium of Medanense, Department of Biology, Faculty of Science, Universitas Sumatera Utara, Indonesia.

Extract preparation

M. pendans leaves were dried in oven at 60°C for 48 h and grounded in an electric blender. The powder was extracted with water by infundation. The extract was evaporated by using a rotary evaporator and kept at 4°C. The extract then called Ant Nest Water Extract (ANWE).

Capsule preparation

The ANWE mixed with maydis starch to obtain a homogeneous mass. The mass was dried in oven at 55°C and then filled into 0-sized capsule shells using a semi-automatic capsule filling device.

Experimental design

This is an experimental study with randomized pre- and post-test group design. The study was conducted from February to November 2018 at Pharmacy Faculty, and Medical Faculty of Universitas Sumatera Utara (USU), USU General Hospital and Integrated Laboratory of Medical Faculty USU.

Volunteer

The volunteer recruitment process was done by announcing the study in USU area. Twelve volunteer involved in this study. Each was explained the study protocol and asked to sign the informed consent before data collection. The inclusion criteria are volunteer who has no smoking, medication, gastritis, and allergic history, with a normal liver (Serum Glutamic-Oxaloacetic Transaminase (SGOT) and Serum Glutamic-Pyruvic Transaminase (SGPT)) and kidney (Ureum (Ur) and Creatinine (Cr)) function. Volunteer that shows any adverse effect or subjective symptoms during the experiment would be excluded from the study.

Intervention

Twelve volunteers were involved in this study. Each volunteer given the capsule once daily for 28 days. No medication was allowed except under exceptional conditions only with the permission of the investigator. There was no restriction placed on normal and routine activity or diet during the study period.[17]

Evaluation of ANWEC on hematology profiles, liver and kidney function, lipid profile and blood glucose level

All volunteers who received Ant Nest Water Extract Capsule (ANWEC) were evaluated of their blood. Laboratory tests (including hematology and blood chemistry) were performed in laboratories at USU General Hospital at baseline and after 28 days of treatment using standardized procedures.

Hematology tests include hemoglobin (Hb), leucocyte and thrombocyte. Blood chemistry tests include SGOT, SGPT, total cholesterol (TC), triglycerides (TG), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Ur and Cr.[19]

Evaluation of ANWEC on MDA level

Twelve volunteers were divided into 2 groups, 6 volunteers in each group. Group I: Ant Nest Water Extract Capsule (ANWEC) in dose of 350 mg ANWE, and group II: Placebo Capsule (PC) which contained 350 mg amylum. Each group given the capsule once daily for 28 days. Malondialdehyde level was measured at day-28 which was the last day of the intervention using MDA (*Malondialdehye*) ELISA Kit MFR: Elabscience. The comparison was made between group I and group II.

Statistical analysis

All the values are represented as mean ± SD. Data were analyzed by IBM SPSS Statistics 22 software and the group means were compared by Mann-Whitney U and Wilcoxon Signed Ranks Test. A probability of $p < 0.05$ was considered as significant.

Ethical clearance

Protocol has been approved by Health Research Ethical Committee (No. 422/TGL/KEPK FK USU-RSUP HAM/2018).

RESULTS AND DISCUSSION

Herbal, botanical, or phytomedicines are medicinal products containing active ingredients of particular plant origin.[20] Many herbal mixtures are already commonly used and expand rapidly across the world, either for primary health care or as complementary or alternative medicines.[21,22] Although herbal medicine have been perceived by the public as relatively low risk, there has been more recognition of their potential risks. Potential harm can occur via inherent toxicity of herbs and or interactions with other herbal products or conventional drugs.[23] Therefore, since safety being a major issue of using herbal medicine [24] unless being supported with essential data including the safety information of body organ systems, herbs are not likely to become an important alternative standard medical therapies.[25]

Before a drug comes to market, it is studied in clinical trials in healthy and particular selected disease as the efficacy investigated.[26] It is known that after an administration of a drug, it will be absorbed to reach systemic circulation, followed by distribution, metabolism or biotransformation, and excretion. Drug elimination is usually divided into two major components, biotransformation and excretion. Biotransformation or drug metabolism converts the drug in the body to a metabolite. Most drugs must pass through the liver, as the primary site for drug metabolism. Nonvolatile drugs are excreted mainly by renal excretion, a process in which the drug passes through the kidney to the bladder and ultimately into the urine. Other pathways for drug excretion may include the excretion of drug into bile, sweat, saliva, milk (via lactation), or other body fluids.[27] Additionally, there are many patient factors that influence the pharmacokinetics of a drug, such as age, physiological stress, the presence of other diseases, liver and kidney function.[28] There are attempts for a medicine to get a better handle on the safety profiles before marketing.[26]

Safety

This study used Ant Nest in the form of water extract formulated in capsules (ANWEC) at a dose of 350 mg which is known from the previous study that 350 mg water extract of Ant Nest in capsules once a day for 28 days does not affect blood profile, lipid profile, liver function and kidney function.

Safety test result of Ant Nest Water Extract with a dose of 350 mg in capsule can be seen in Table 1.

Statistically, the *p* value showed >0.05 which means no significant different before and after intervention either within or between group. The table shows no significant difference in the parameter values observed on day-0 and day-28. All parameters are still in the normal range. There were no significant complaints made by volunteers during the treatment. The result showed that there was no side effect of ANWEC in healthy volunteers. Therefore, it might be concluded that ANWE has no toxicity in healthy volunteers.

Therefore, this study suggest that ANWEC at dose 350 mg in capsul safe to be used in healthy human.

Efficacy

Malondialdehyde (MDA) level was measured in the present study to evaluate the potency of *M. pendans* extract as antioxidant agent. Higher levels of MDA concentration has been reported in different studies of diseases. MDA is the major metabolite of arachidonic acid and serves as a reliable biomarker for oxidative stress. MDA is a mutagenic, tumorigenic and highly reactive three-carbondialdehyde produced during polyunsaturated fatty acid peroxidation and arachidonic acid metabolism.[29] Determination of MDA in blood plasma or tissue homogenates is one of the useful methods to predict the oxidative stress levels.[30] The MDA level test was taken on day-28 and the results are provided in Table 2.

It can be learned from Table 2 that MDA level in ANWEC-treated group was 117.2± 23.8 which means lower than placebo (147.25 ± 18.7 µg/mL). The *p* value <0.05 indicates that there is a significant difference between groups. This result support the previous preclinical studies that reported the antioxidant activity of this plant.[15,16,17,18] It was also showed the future opportunity of this plant as a source for antioxidant efficacy. However, this result still need to be verified with more number of subjects.

Table 1: Evaluation of ANWEC on hematology profiles, liver and kidney function, lipid profile and blood glucose level

Parameters	Results (Mean + SD)		*p*
	Day 0	Day 28	
Hematology			
Hb (g/dL)	14.33 ± 1.01	14.15 ± 1.32	0.68
Leucocyte (*1000/uL)	6.99 ± 0.81	6.76 ± 1.32	0.75
Thrombocyte (*1000/uL)	282.50 ± 55.86	28.17 ± 46.34	0.67
Liver			
SGOT (U/L)	22.67 ± 4.46	21.50 ± 3.39	0.40
SGPT (U/L)	18.17 ± 5.31	19.50 ± 6.59	0.29
Kidney			
Ur (mg/dL)	19,42 ± 4,13	18.80 ± 2.78	0.60
Cr (mg/dL)	0,97 ± 0,19	1.06 ± 0.18	0.05
Lipid Profile			
TC (mg/dL)	160.97 ± 27.56	158.80 ±30.79	0.60
LDL (mg/dL)	109.66 ± 29.52	107.50 ±42.33	0.91
TG (mg/dL)	96.33 ± 35.41	88.67 ± 18.06	0.91
HDL (mg/dL)	58.33 ± 15.14	52.11 ± 11.03	0.07
Glucose			
Fasting blood glucose level (mg/dL)	89.5 ± 8.73	95.33 ± 6.56	0.13

Table 2: Evaluation of ANWEC on Malondialdehyde Level

Group	MDA (ng/mL)
ANWEC	117.2 ±23.8
PC	247.25 ±18.7
p	0.034

CONCLUSION

It can be concluded that the consumption of Ant Nest Water Extract dose of 350 mg in capsule for 28 days was safe to be used in healthy volunteers with an antioxidant activity proved by the decrease in MDA level.

ACKNOWLEDGEMENT

The study was supported by Ministry of Research and Technology and Higher Education Republic of Indonesia, Research and Community Service, Universitas Sumatera Utara, DRPM2018: No.263/UN5..2.3.1/PPM/KP-DRPM/2018

CONFLICTS OF INTEREST

No conflicts of interest to be declared by authors.

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GRAPHICAL ABSTRACT

Ant Nest (*Myrmecodia pendans)* Water Extract

Capsule of ANWE

Human Study:
n = 12 subjects
Dose 350 mg/ day
28-day duration

Parameters	Results (Mean + SD)	
	Day 0	Day 28
Hematology		
Hemoglobin (Hb)	14.33 ± 1.01	14.15 ± 1.32
Leukosit	6.99 ± 0.81	6.76 ± 1.32
Trombosit	282.50 ± 55.86	28.17 ± 46.34
Liver		
SGOT	22.67 ± 4.46	21.50 ± 3.39
SGPT	18.17 ± 5.31	19.50 ± 6.59
Kidney		
Ureum	19.42 ± 4.13	18.80 ± 2.78
Kreatinin	0.97 ± 0.19	1.06 ± 0.18
Lipid Profile		
Total Cholesterol	160.97 ± 27.56	158.80 ±30.79
Low Density Lipoprotein (LDL)	109.66 ± 29.52	107.50 ±42.33
Trigliserida	96.33 ± 35.41	88.67 ± 18.06
High Density Lipoprotein (HDL)	58.33 ± 15.14	52.11 ± 11.03
Glucose		
Fasting Glucose Level	89.5 ± 8.73	95.33 ± 6.56

ABOUT AUTHORS

Tri Widyawati is a Lecturer at Medical Faculty, Universitas Sumatera Utara, Medan, Indonesia since 2003. Her PhD was graduated from Pharmaceutical Sciences University Sains Malaysia 2016. Currently, besides being a lecturer she is also the head of the Pharmacology and Therapeutic department in her institution. Her research interest is in pharmacological activity of medicinal plant. Currently she is studying the potential effect of active compound as antidiabetic and herbs combination as an immunomodulator.

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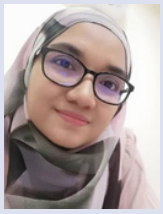
Imam Bagus Sumantri, is a lecturer and researcher at Department of Biology Pharmacy, Faculty of Pharmacy, Universitas Sumatera Utara. His current position is Head of Biology Pharmacy Laboratory, and his research fields focused on Biology Pharmacy such as Pharmacognosy, Traditional Medicine and Phytochemistry.



Nor Adlin Md Yusoff is a senior lecturer at the Cluster of Integrative Medicine of the Advanced Medical and Dental Institute. She received her Ph.D. in Pharmacology in 2017 from Universiti Sains Malaysia. Her research is situated in the field Pharmacology, with a special focus on the efficacy of herbal medicines as a therapeutic agents for metabolic syndromes, particularly diabetes mellitus. Currently, she is studying the potential effect of phytochemicals in the modulation of glucose transporters. Nor Adlin teaches several courses for Master of Health Toxicology programme. She is also a peer reviewer for several citation indexed journals.

Cite this article: Widyawati T, Pase MA, Daulay M, Sumantri IB, Yusoff NA. Evaluation of *Myrmecodia pendans* Water Extracts on Hematology Profiles, Liver, Kidney Function and Malondialdehyde Level in Healthy Volunteer. Pharmacogn J. 2020;12(6) Suppl:1489-93.