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Comparative Effect of Yellow Root (*Arcangelisia Flava* (L) Merr) Water and Brackish Water Decoction on Biochemical Profiles of Renal Function and Histopathological Profiles of Renal and Uterus in Wistar Female Rats

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ABSTRACT

The yellow root (*Arcangelisia flava* (L) Merr) is a medicinal plant used traditionally in Borneo, Indonesia for several diseases but on the other hand it contains berberine alkaloids considered as harmful compounds and it has been banned in some ASEAN region for traditional medicines. Empirically, as local wisdom, the people do not boil yellow roots with water but with brackish water. This study aims to determine the effects of yellow root decoctions with water and those with brackish water on blood biochemical and histopathological profile of renal and uterus of Wistar rats after subchronic treatment. A total of 70 Wistar female rats were divided into 7 groups, 3 groups were given yellow root water decoctions and 3 groups were given yellow root brackish water decoctions with doses equivalent to 1.25, 2.5 and 5 g/kgBW of dried yellow root respectively, while the control group was only given aquadest. After 28 days of treatments blood samples were obtained for biochemical examination followed by surgical examination to see the histological features of different organs. The results showed that there were significant differences on the data of blood urea nitrogen (BUN) levels of water decoction of yellow root in comparison to normal group while those of brackish water decoction were not significant except the biggest dose. The histopathological features of rats treated by all doses of the yellow root water decoctions showed organ damage compared to normal group while only the highest dose of the yellow root brackish water decoction gave the damages shown by, hemorrhage, degeneration and necrosis of the kidney as well as epithelial damage and inflammatory infiltration of the uterus. So the result support the decision of ASEAN Traditional Medicine and Health Supplement Scientific Committee to inhibit the use of Arcangelisia flava in traditional medicine but on the other hand the people can still use the brackish water decoction as local wisdom.There is no significant different on berberin contents of the water and brackish water decoction so the different effects are not directly correlated to berberine contents.

Key words: *Arcangelisia flava*; subchronic renal and uterus toxicity; berberine

INTRODUCTION

Indonesia is an archipelago in the world with more than 13,000 island and consists of hundreds tribes. In Central Borneo there is a tribe namely Dayak that known having some specific local wisdom including their traditional medicines. Since the past traditional medicines have great benefits in maintaining health and treat disease, so until now traditional medicine is still often used by the community. One of the medicinal plants cultivated in Borneo island is yellow fruited moonseed (*Arcangelisia flava* (L.) Merr.) that popularly also named yellow root. It belong to Menispermaceae family and empirically the people use to treat jaundice, smallpox, digestive problems, worm, fever, menstruation disorder and sprue (Kardono *et al.,* 2003, Keawpradub, 2005). Wu *et al*, (2006) reported that water extract of yelow root with dose 150 mg / kg BW showed hepatoprotector activity and antiplasmodial activity. On the other hand yellow root contains berberine alkaloids that are considered as harmfull compounds. Berberine has been shown to be safe in the majority of clinical trials. However, there is a potential for interaction between berberine and many prescription medications. Berberine is not recommended in pregnant or breastfeeding women due to a lack of available scientific evidence. Although not well studied in humans, berberine has been suggested to have anti-fertility, abortifacient (abortion inducing), and uterine stimulant activity. Berberine may cause abortion, eye or kidney irritation, nephritis (inflamed kidneys), dyspnea (difficulty breathing), flu-like symptoms, giddiness, and lethargy (ATSC, 2017).

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Based on the harmfull effect of berberine it will be urgent to determine the safety of the use of yellow root containing berberine especially in rennal function and uterus. Interestingly that Dayak people use brackish water decoction instead of water decoction in preparing remedies containing yellow root so it will be important to compare the effect of these two decoction of yellow root after subchronic oral treatment.

METHODOLOGY

Yellow root

Yellow root used in this research was collected from Central Borneo Province. Authentication of yellow roots was done in Biology Laboratory Faculty of Mathematics and Natural Sciences Ahmad Dahlan University Yogyakarta.

The preparation of yelow root decoctions

The yellow roots were weighed and were divided into two parts. The first one was boiled with aquadest and the second was boiled with brackish water, for 30 minutes then filtered and ready to be determined their subchronic toxicity and berberine levels..

Animal Testing

In this study we used female Wistar rats aged 1.5 months with an average weight of 150-200 grams, as many as 70 randomly divided into 7 groups so each group consisting of 10 rats.
Normal Control group I: aquadest; Group II: yellow root water decoction dose of 1.25 g / kgBB; Group III: yellow root water decoction, dose of 2.5 g / kgBB; Group IV: yellow root water decoction, dose of 5 g / kgBB; Group V: yellow root brackish water decoction, dose of 1.25 g / kgBB; Group VI: yellow root brackish water decoction,, dose of 2.5 g / kgBB; GroupVII: yellow root brackish water decoction,, dose of 5 g / kgBB.
Rats were placed in individual cage and stuckgiven feed and drinking water ad libitum

Subchronic toxicity test

The research has got an agreement from Universitas Gadjah Mada Ethical Commision with number 011703033 on May 30th 2017 and the subchronic toxicity test was performed according to procedure established by National Agency for Drug and Food Control of Republic Indonesia (Anonymua, 2014).. The yellow root decoctions were performed for 28 days, and then observed daily to determine the presence of behaviour toxicity, and at the end of experiment the blood was collected from sinus orbitalis for biochemical examination and then performed surgery to see the histopathological features of the organs. Biochemical examination of renal function included blood urea nitrogen analysis was done at the Central Research Laboratory (LPPT) whereas histopathological analysis was done at Laboratory of Pathology and Anatomy Faculty of Medicine Universitas Gadjah Mada Yogyakarta. The organs observed were kidney and uterus. For histopathologic examination organ samples were fixed in a 10% normal formaldehyde buffer solution (BNF), dehydrated with alcohol of various concentrations, clearing with xylol and embedded in paraffin. The tissue samples were cut with a thickness of 5 μm and the preparations were stained with eosin hematoxylin (HE). Microscopic observations were performed with an objective lens 40 times. Observations were made in 5 fields of view (Kieman, 2001).

Statistical analysis

The result of this research was quantitative data of biochemical profile and scoring data of organ histopathology damage of yellow root brackish water decoction groups which then compared to control group and yellow root water decoction groups were analyzed statistically using SPSS program. The data obtained statistically using Kolmogorov-Smirnov Test and Homogeneity of Variance Test to determine the normality and homogeneity of the data obtained. Then the test continued with one way ANOVA parametric analysis. If there is a significant difference test followed by LSD with 95% confidence level.

Determination of berberine

High performent thin layer chromatography (HPTLC) method was used to determine berberine levels in the yellow root decoctions. Silica gel F254 HPTLC plates were used as stationary phase and the upper phase of *n*-buthanol-acetic acid-water (4:1:5 v/v) was used as optimized mobile phase. Linier curve of different concentrations of standard reference of berberine was used to calculated berberine contents in the decoctions.

RESULTS AND DISCUSSION

Subchronic Toxicity Test

The result of biochemical tested parameters are mentioned in table I.

Doses of yellow root decoction with both water and brackish water may lead to anatomic pathological changes in some organs such as kidneys and uterus

Data of table I showed that there were statistically and clinically significant different between BUN levels of rat groups treated with yellow water decoction in comparison to those of normal control group, while the treatment of

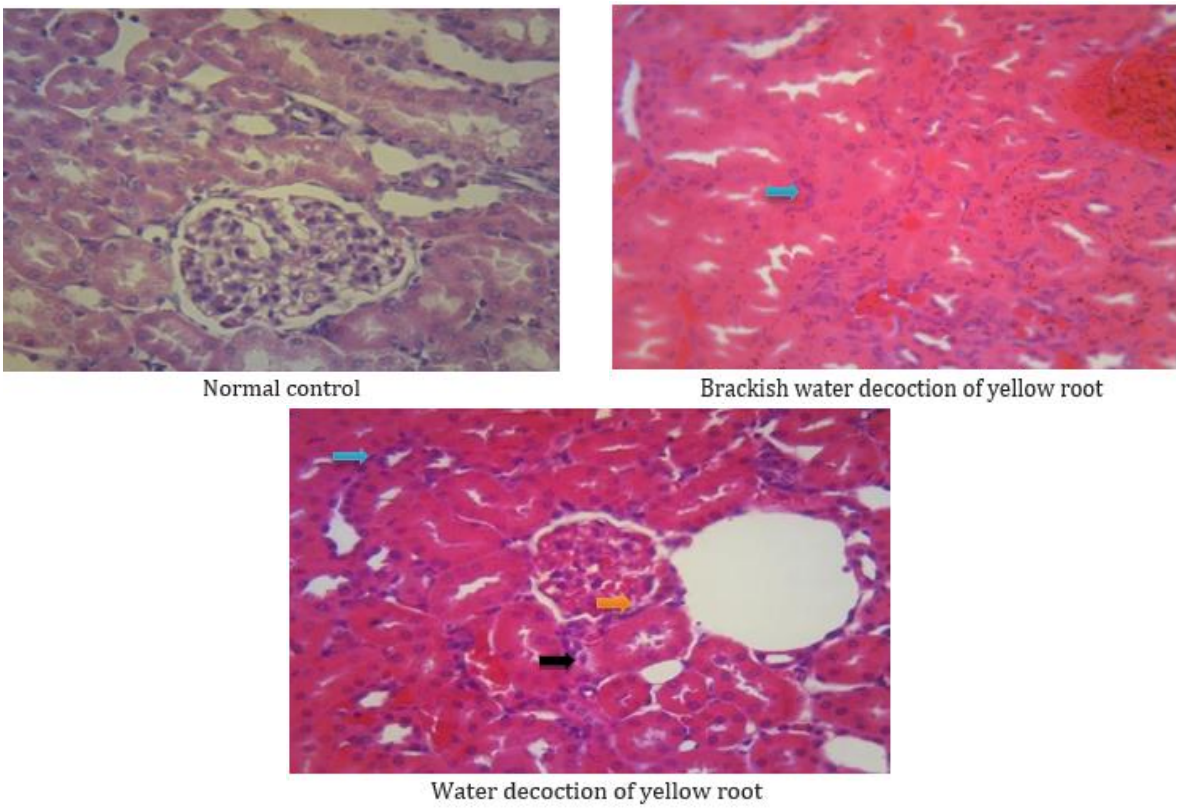

Figure 1. Histopathological features of renal organs: normal control group, yellow root brackish water decoction group dose 5 g / kgBW, yellow root water decoction dose 5 g / kgBB (B): hemoraghi (➡), degeneration (➡) and necrosis (➡)

Table I. Blood Biochemical Levels of Female Rats Treated with Yellow Root Water and Brackish Water Decoction for 28 Days

Groups	BUN (mg/dL)	CREATININE (mg/dL)
Normal Control	29.02 ± 4.25	0,45 ± 0,11
Water Decoction		
1.25 g/Kg BW	45,72 ± 7,09*	0,39 ± 0,10
2.5 g/Kg BW	48,46 ± 7,26*	0,36 ± 0,05
5,0 g/KgBW	43,90 ± 3,16*	0,35 ± 0,07
Brackish Water Decoction		
1.25 g/KgBW	25,32 ± 3,94	0,48 ± 0,04
2.5 g/KgBW	29,18 ± 6,62	0,42 ± 0,08
5.0 g/KgBW	25,32 ± 1,73	0,43 ± 0,08

*significant difference ($p<0.05$) in comparison to normal control group.

brackish water decoction did not cause the increase of BUN levels. These results were in line with histopathological data of kidney shown in figure 1.

Figure 1 shows that there is blood capillary damage so that blood escapes from the blood vessels and causes hemorrhage and then degeneration damage and cells undergoing necrosis which begins with morphological changes in the nucleus of the next stage of the nucleus (the cariesection) and the nucleus disappears (carriolisis). It can be concluded that the treatment of rats with yellow root water decoction caused damage on kidney at all dose given, while only dose of 5 g/kgBW of brackish water decoction caused damage on the organ tested. It must be noted that the kreatinine levels were not changed.

The data of uterus can be seen table II and figure 3.

Table II shows a marked difference, meaning that there is damage in the histopathology

Table II. Scoring microscopic view and rats organ ratio of uterus

Groups	Scoring microscopic view of Uterus	Rats organ ratio
Normal Control	Normal	0,898 ± 0,04
Water Decoction		
1.25 g/Kg BW	Normal	0,986 ± 0,05*
2.5 g/Kg BW	1,47±0,3*	0,968 ± 0,06*
5,0 g/KgBW	2,80±0,2*	1,149 ± 0,09*
Brackish Water Decoction		
1.25 g/KgBW	Normal	0,897 ± 0,09
2.5 g/KgBW	Normal	0,906 ± 0,08
5.0 g/KgBW	2,40±0,3*	1,005 ± 0,08*

Description: * there is a noticeable difference (P <0.05)

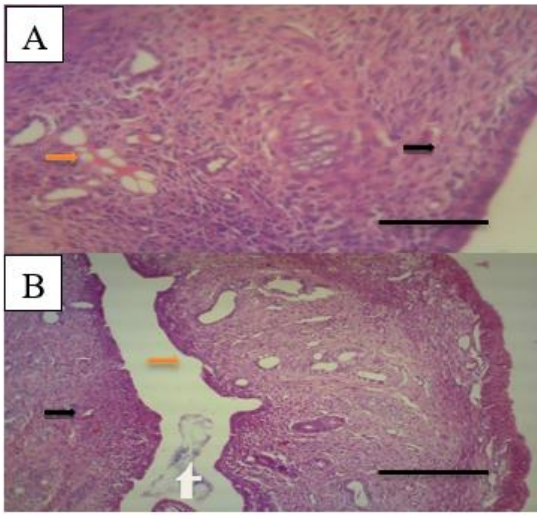

Figure 2. Histopathological features of the yellow root brackish water decoction of 5 g/kgBW (A) and yellow root water decoction of 5 g / kgBB (B), Seen from epithelial neoplasi (➡) and inflammatory infiltration (➡) with HE staining and 400x magnification increased, Bar = 200µm

of uterine organ of the water decoction group dose 1.25g / kgBB, 2.5g / kgBB and 5g / kgBB, and only of the brackish water decoction group, dose 5g / kgBB. So it can be seen that the damage caused by water decoction group is greater than the brackish water decoction group.

Figure 2 shows that the presence of epithelial cells present in the lumen part is the release of the epithelial mucosal part, resulting in the epithelium becoming thinner. Epithelial cells in the endometrium area are seen lysing, vascularization of the tissue, where the epithelium is attached to the gland so that the lamina propia becomes thin and epithelial neoplation occurs ie the growth of new epithelial tissue, infiltration of inflammatory cells caused by inflammation caused by damage tissue and may be lymphoid follicle cells, as well as seen in uterine changes including epithelial hyperplasia, cilia loss, tubular glandular atrophy, degeneration and epithelial desquamation (Gibson, 2003, Saruhan *et al.,* 2006).

Berberine content

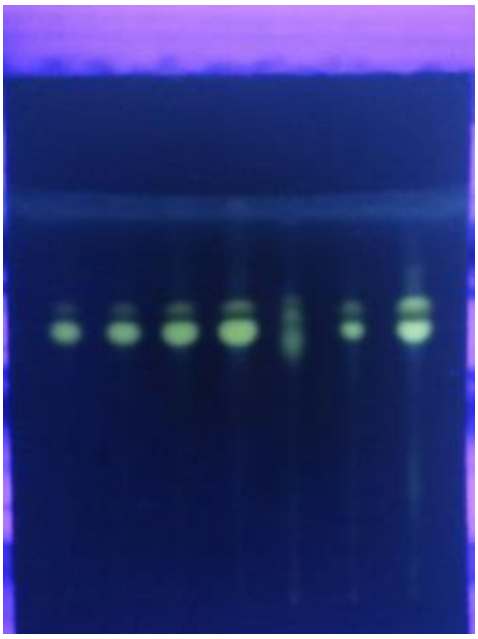

Figure 3. HPTLC profile of berberine standard reference, water and yellow root brackish water decoction on Silica F_{254} and elution with the upper phase of *n*-buthanol-acetic acid-water (4:1:5 v/v).

HPTLC – densitometry analysis showed that There is no significant different on berberin contents of the water and brackish water decoction so the different effects are not directly corelated to berberine contents. It is possible that there was influence of brackish water on the absorbsion of berberine.

CONCLUSION

The results showed a significant difference in effect of all dose given of yellow root water decoction group with (P <0.05) which tend to be

toxic, while only the bigest dose of brackish water decoction caused the damage. With the appearance of hemorrhage, degeneration and necrosis of the kidney as well as epithelial neoplasi and inflammation of the uterus.

REFERENCES

National Agency for Drug and Food Control Republic IndonePublisher BPOM, Jakarta..

ASEAN Traditional Medicines and Health Supplements Scientific Committee, ATSC, 2017, The ASEAN List for Traditional Medicines and Health Supplements (TMHS) containing active substances which are subject to specific restrictions for use in at least one of the ASEAN Member States,.

Gibson J. 2003. Physiology and Modern Anatomy. Jakarta: EGC.

Keawpradub, N., Dej-adisai, S., and Yuenyongsawad, S., 2005, Antioxidant and Cytotoxic Activities of Thai Medicinal Plants Named Khaminkhruea: Arcangelisiaflava, Cosciniublumeanum, and Fibraureatinctoria., Songklanakarin J. Sci. Technol., 27 (Suppl.2): 455-467

Kardono LBS, Artanti N, Dewiyanti ID, Basuki T, Padmawinata K. 2003. Selected Indonesian Medical Plant Monograph and Description. Jakarta. PT GramediaWidiasarana Indonesia.

Kieman, J.A., 2001, Histological and Histochemical Methods, 3rd Ed., Toronto, Arnold Pub, 330-354

Saruhan, B.D., Ozbag, G., Ozdemir, N. & Gumusalan, Y. (2006). Comparative Effect Ovariectomy and Flutamin on Body Uterus Ovariectomized Rat Model. J. Inonu Med. 13, 221-226.

Wu X, Li Q, Xin H, Yu A, et al., (2006). Effect of berberine on the blood concentration of cyclosporin A in renal transplanted recipients, clinical and pharmacokinetic study; Eur J Clin Pharmacol 61(8):567-572.