

Subchronic Effect of Yellow Root (*Arcangelisia Flava* (L) Merr.) Containing Berberine Boiled With Water and Brackish Water On Hematological, Blood Biochemical and Histopathological Parameters of Liver Function In Female Wistar Rats

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Yellow fruited moonseed (*Arcangelisia flava* (L) Merr.) from *Menispermaceae* family is a kind of plant which has been used traditionally by people in Borneo island and its root is popularly called yellow root. The people use the root as a tonic, as an alternative medicine for hepatitis, diarrhea, and skin diseases (Keawpradub et al., 2005). Furthermore yellow root contains berberine alkaloid as bioactive compound. Berberine has been reported that it reduced heart disease or non alcoholic fatty liver disease (NAFLD). It has a role in heart steatosis recovery, lipid metabolism disturbance, inflammation relieve, and insulin resistance (Xing et al., 2011). On the other side, berberine is considered as a harmful compound. Berberine is not only provided on the yellow root plant, it can also be found in *Hidrastis canadensis*, *Coptis chinensis*, *Berberis aquifolium*, *Berberis vulgaris*, and *Berberis aristata*. All these plants are in the ASEAN restricted list of active ingredients (ATSC, 2017) and the containing products are not allowed in some ASEAN member countries including Indonesia. In Philippines the

ABSTRACT

Objective of this research is to examine the subchronic effect of yellow root water and brackish water decoction administered to female Wistar rats. Methods, 70 rats were divided into 7 groups: the first group was treated with water as normal control; the second, third and fourth groups were treated with water decoction of yellow root; the fifth, sixth and seventh groups were treated with brackish water decoction of yellow root, 1.25 g/KgBW, 2.5 g/KgBW and 5g/KgBW respectively. The decoctions were given orally every day for 28 days. Hematological, general blood biochemical, close related histopathological and biochemical parameters of liver function were determined. HPTLC Densitometry method was used to determine the berberine contents on yellow root decoctions. The results showed that the treatment of all doses of yellow root water decoction statistically and clinically increased significant SGPT levels and interestingly they were in line with microscopic view of liver even only steatosis condition. It must be noted that the SGPT levels of rats treated with yellow root water decoctions were 64.04, 59.36 and 68.12 U/L. They were significantly very high in comparison to normal range levels (16-48 U/L). The berberine contents of the yellow root water decoction were 0,99% while those of yellow root brackish water decoction were 1,38%. Yellow root water decoction had toxic effect in liver function while yellow root brackish water decoction did not cause toxic effect after 28 days treatment in female Wistar rats.

KEYWORDS: Subchronic toxicity, hematological, liver function, berberine, *Arcangelisia flava*

1. Introduction

People has known and used the plant as an alternative medicine to prevent the health problem.

products containing berberine require warning statements as follow: Consult your practitioner if you have conditions such as Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency, haemolytic anemia, glaucoma, diabetes, high blood pressure, history of cardiovascular disease or if you are using paclitaxel, cyclosporin or other chemotherapeutic agents. Not to be taken by: babies, children under 12 years of age, pregnant women or lactating mothers.. One kind of plant which contains berberine is *Coptis chinensis*. It belongs to neonatal jaundice herbal healing in China, and a case study showed there was unlucky baby from a mother who has consumed the herbal during the pregnancy having more serious in hiperbilirubinemia than baby whose mother didn't consume that herbal medicine (Fok et al., 2001). Furthermore it was reported that the symptoms of poisoning of other plant containing berberine namely *Berberis vulgaris* L. were characterized by lethargy, stupor and daze, vomiting and diarrhea, and nephritis (Wolters Kluwer Health, 2005).

The benefits of traditional medicine are caused by chemical compound inside, but on the other side it also can cause the toxic effect. In Indonesia, berberine belongs to the forbidden chemical compounds thus it could not be used as an ingredient of a medicine registered to Indonesian FDA. Based on that argument the yellow root as a traditional medicine should be examined in order to evaluate the safety of this plant material while it still be traditionally used by the people. Interestingly, the Dayak tribes in Central of Borneo use yellow root brackish water decoction instead of water decoction. In order to check the safety of traditional medicine used by the people it will be very important to examine then analyze the toxic level of yellow root brackish water decoctions given subchronically on female rats in comparison to those of yellow root water decoction and also to determine the berberine contents.

2. Materials and methods

2.1 Materials

The sample used is yellow root, water and brackish water collected from Central Borneo area. Berberine reference standard was obtained from Sigma Chemical Co. (St. Louis, MOUSA). 60 F254 silica gel HPTLC plate, *n*-butanol, glacial acetic acid was obtained from E. Merck (Germany).

2.2. Preparation of samples

The sample was made by boiling 1 part of root of the plant in 10 part of water and in brackish water separately. It was made based on the certain concentrations from the empirical dosages. After getting the decoctions, the sample was ready to be tested to the rats orally. In order to inhibit microbial contamination, the decoctions were stored in refrigerator and they were warmed every day before administered to the rats.

2.3. Subchronic toxicity test.

The research has got an agreement from Universitas Gadjah Mada Ethical Commission with number 011703033 on May 30th 2017. Subchronic toxicity test was conducted based on the procedure of OECD (2008). 70 female Wistar rats of 1.5 months old, 120-200 g in weight were divided into 7 groups: the first group was treated with water as normal control; the second, third and fourth groups were treated with water decoction of yellow root; the fifth, sixth and seventh groups

3. Results and discussion

3.1 Effect on blood biochemical parameters

The results of several blood biochemical and hematological measurements are presented in Table 1 and Table 2, while data of liver function, i.e SGPT, SGOT and bilirubin are presented head to head with histopathological data in Table 3 in order to look at the correlation.

Table 1 Blood Biochemical Parameter Levels of Female Rats Treated with Yellow Root Water and Brackish Water Decoction for 28 Days

Groups	Cholesterol	Albumin	Glucose	Protein
Normal Control	65.98 ± 4.71	3.73 ± 0.27	91.14 ± 1.00	8.32 ± 0.57
Water Decoction 1.25 g/Kg BW	46.82 ± 5.46*	4.08 ± 0.30	86.20 ± 1.45*	7.22 ± 0.39*
2.5 g/Kg BW	46.10 ± 8.18*	4.08 ± 0.38	85.62 ± 1.64*	7.22 ± 0.33*
5,0 g/KgBW	49.96 ± 5.91*	4.08 ± 0.30	90.46 ± 4.91	7.34 ± 0.32*
Brackish Water Decoction 1.25 g/KgBW	48.32 ± 8.36*	3.54 ± 0.28	82.60 ± 7.88	8.06 ± 0.24 [^]
2.5 g/KgBW	56.76 ± 7.26*	3.95 ± 0.18	96.54 ± 8.68	8.40 ± 0.22 [^]
5.0 g/KgBW	52.66 ± 7.09*	3.92 ± 0.33	99.94 ± 20.29	8.14 ± 0.61 [^]

* was significantly different than normal control group (p < 0.05)

[^] was significantly different compared to the yellow root water decoction group (p < 0.05)

The data of several blood biochemical parameters presented in Table 1 showed that there were significant different (p < 0.05) on cholesterol levels between all doses of either water and brackish water decoction groups in comparison with normal control

were treated with brackish water decoction of yellow root; 1.25, 2.5 and 5g/KgBW respectively. The choice of female rats was based on the restriction of use of the plants containing berberine. The equipment used in the research are glasses rat cages, rat drinking place, rat weight scale, hotplate, oral tool, micropipette, microtube, centrifuge, Biolyzer 100TM, rotavapor, and densitometer. Blood biochemical 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. Animals were weighed, as well as observation of toxic symptoms and clinical symptoms in the form of change of feathers, secretions, excretions, and other activities, performed daily for 28 days. After 28 days of treatments with water decoction and brackish water decoction blood biochemical and hematological analysis were done and then after sacrifice histopathological analysis were performed. The data of the research were analyzed using SPSS program which included normality, homogeneity, one way Anova test and LSD test to determine the significant difference of 7 treatment groups.

2.4. Berberine analysis

The sample was 10 mL of yellow root water decoction and brackish water decoction. By using separated funnel it were then shaken with ethyl acetate in order to dissolve berberine contained in the decoctions. Quantitatively 5 µL of the ethyl acetate fractions were spotted on a 60 F254 silica gel HPTLC plate using a micropipette. After that, berberine reference standard stock solutions were made with methanol to obtained 0.03125%, 0.0625%, 0.125% and 0.250% and they were also spotted on a pre-prepared HPTLC plate of 5 µL. The plate was inserted into chamber for elution with upper phase of *n*-butanol-glacial acetic acid-water (4:1:5) as mobile phase. The plate was observed under UV light of 366nm. Then the areas (AUC) were measured triplicate with on Camag TLC Scanner at maximum wavelength operated by CATS software (VI.2.6, Camag). The regression of standard curve was calculated with equation $y = bx + a$, with *x* as the berberine content and *y* as the area. So the berberine levels in the sample can be calculated. The correlation between berberine content and the toxicological effects was subject to be discussed.

group. The obtained data is in line with previous research on the hypolipidemic effect of berberine with the mechanism of increasing the number of cell receptors (upregulating) of the expression of low-density lipoprotein receptor (LDLR). LDLR itself is a receptor responsible for regulating the (homeostatic) balance of LDL-cholesterol in plasma located in human hepatocyte cells (Hu, et al., 2012; Kong, et al., 2008; Wu, et al., 2012). Even though, based on list of normal blood biochemical levels of female rats established by Giknis and Clifford (2008) the cholesterol level of all treated groups were still in the normal range of cholesterol level of female rats (24-73 mg/dL). About albumin parameters, according to Evans (2002) under normal circumstances, only 20-30% of hepatocytes produce albumin, but the production rate of the albumin varies depending on the state of the disease and the rate of nutrition because albumin is only formed in the appropriate osmotic, hormonal and nutritional environments. In addition, the colloidal osmotic pressure of the interstitial fluid that soaks up the hepatocytes is an important albumin synthesis regulator. There were no significant different on albumin levels in this research. Furthermore, there were two doses of water decoction groups that caused decrease of glucose levels ($p < 0.05$) In relation to the glucose parameter, basically the berberine compound contained in the yellow root works in inhibiting the absorption of glucose in the intestinal, so that berberine can produce a glucose lowering effect (Pan, 2003). Data of Table 1 showed that all glucose levels were still in the normal range (76-175 mg/dL). The protein levels of all doses of water decoction groups were significantly decrease in comparison to control group and brackish water decoction groups. Even though the levels were still within the normal range of protein level of female rats (5.5-7.7 g/dL). Based on the data mentioned in Table 1, it can be concluded that the treatment of yellow root water decoction trend to change negatively all blood biochemical parameter levels in comparison to those of normal control group, while the treatment of brackish water decoction did not change the parameters except for cholesterol levels. The two decoctions decrease cholesterol levels but the levels were still in normal range. In point view of toxicity the two decoctions of yellow root are not categorized as harmful substances.

3.2 Effect on hematological parameters

Furthermore the data on important hematological examinations such as hemoglobin, erythrocytes and leucocytes are mentioned in Table 2. There were no significant differences between erythrocyte and hemoglobin levels of all dose of water and brackish water decoction of yellow root.

Table 2 Results of Haematological Parameter Levels of Female Rats Treated with Yellow Root Water and Brackish Water Decoction for 28 Days

Groups	Leucocytes ($10^3/\mu\text{L}$)	Erythrocytes ($10^6 \mu\text{L}$)	Hemoglobins (g/dL)
Normal Control	4,20 ± 0,9	4,82 ± 1,6	10,64 ± 2,2
Water Decoction 1.25 g/Kg BW	4,12 ± 0,8	5,30 ± 0,3	11,10 ± 0,7
2.5 g/Kg BW	4,14 ± 1,0	5,65 ± 0,7	11,84 ± 1,5
5,0 g/KgBW	4,16 ± 1,0	6,05 ± 0,7	12,12 ± 1,4
Brackish Water Decoction 1.25 g/KgBW	4,52 ± 1,2	3,26 ± 1,8	6,72 ± 2,6
2.5 g/KgBW	3,12 ± 1,1*	4,05 ± 1,2	9,26 ± 2,1
5.0 g/KgBW	2,56 ± 0,8*	4,45 ± 1,3	10,00 ± 1,6

* was significantly different in comparison to normal control ($p < 0.05$)

The interesting result was in the data of leucocyte levels. The treatment with 3 doses of water decoction of yellow root did not practically change leucocyte levels, while 2 doses of brackish water decoction decreased significantly leucocyte levels. It was in line with the research on Coptidis rhizome containing berberine (Linn, et al., 2012). The biggest dose of yellow root brackish water decoction caused the decrease of leucocyte levels until $2.56 \times 10^3 / \mu\text{L}$ that means almost 40% from normal control group and they were doses dependent. In point view of toxicity, the leucocyte levels still in the normal range ($1.13-7.49 \times 10^3 / \mu\text{L}$), while in point view of activity it was interesting because it open the possibility to use yellow root as anti-leucaemia. It will be programmed for future research.

3.3 Effect on liver functionl parameters

The most important result of this research is on the liver parameter levels. The examination of SGPT and bilirubin are the right indicator because they have characteristic and specific to the image of liver cell damage, while SGOT is less specific but still has correlation with liverfunction. Data of liver parameters, i.e SGPT , SGOT and bilirubin are presented head to head with histopathological data in in order to look at the correlation between biochemical and histopathological data such as shown in Table 3 and Figure 1 respectively.

Table 3 Microscopical Data of Liver of Rats Treated with Water and Brackish Water Decoction of Yellow Root for 28 Days, head to head with the SGPT, SGOT and bilirubin levels

Groups	Steatosis	SGPT (U/L)	SGOT (U/L)	Bilirubin (mg/dL)
Normal Control	0,00 ± 0,00	44.76 ± 6.41	108.26 ± 14.96	0.38 ± 0.04
Water Decoction 1.25 g/Kg BW	0,73 ± 0,31*	64.04 ± 6.55*	148.8 ± 43.77	0.33 ± 0.06
2.5 g/Kg BW	1,47 ± 0,23 *	59.36 ± 14.08*	131.5 ± 27.26	0.28 ± 0.06
5,0 g/KgBW	1,80 ± 0,20*	68.12 ± 14.63*	134.18 ± 29.36	0.31 ± 0.03
Brackish Water Decoction 1.25 g/KgBW	0,00 ± 0,00^	39.70 ± 3.16^	82.84 ± 7.89^	0.31 ± 0.05
2.5 g/KgBW	0,00 ± 0,00 ^	42.46 ± 7.66^	83.70 ± 25.79^	0.39 ± 0.05
5.0 g/KgBW	1,13 ± 0,12*^	46.44 ± 9.99^	87.98 ± 18.10^	0.32 ± 0.06

* was significantly different than normal control ($p < 0.05$) ^ was significantly different compared to the yellow root water decoction group ($p < 0.05$)

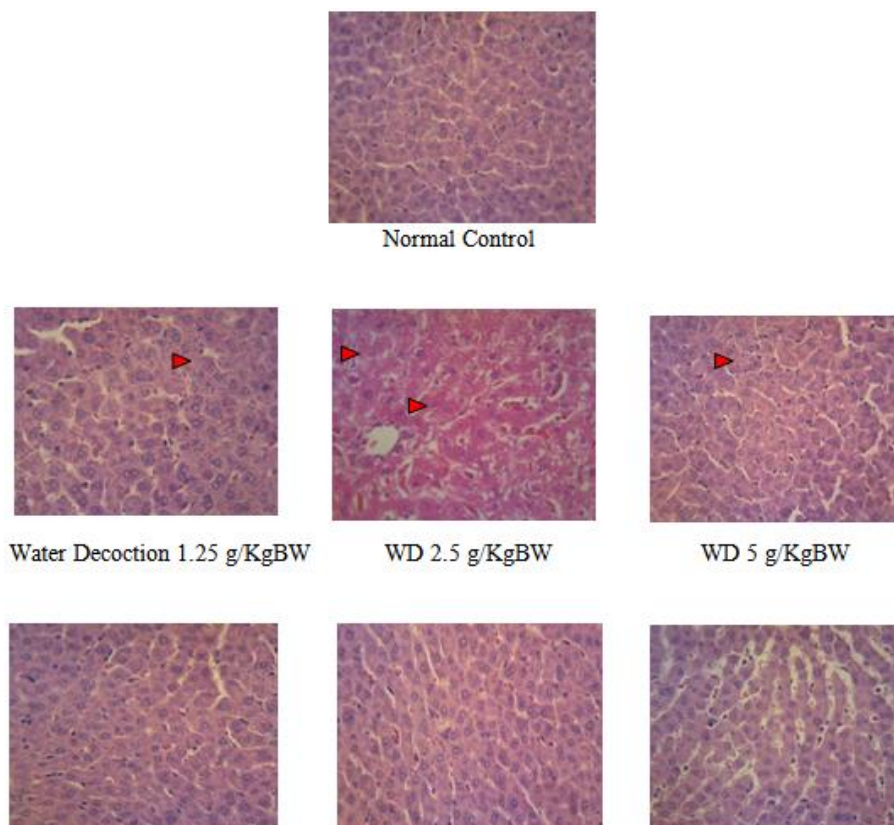


Figure 1. Microscopical View of Liver Histopathological Data of Rats Treated with Yellow Root Water and Brackish Water Decoction for 28 Days, ▶ Steatosis,

Statuses or fatty change of fatty liver is the accumulation of abnormal amounts of lipids in 5% or more hepatic cells. Most steatosis is of the macrovesicular type, in which a single large fat vacuole or several smaller ones occupy the greater part of the cell, pushing the nucleus to the periphery (Pablo, 2012). In this research, microscopical view of liver organ showed steatosis condition especially in rats treated with yellow root water decoction, while the rats treated with yellow root brackish water decoction did not show steatosis, the cels appeared normally.

Data in table 3 showed that there were increase of SGOT levels of rats treated with yellow root water decoction clinically significant but due to relatively big deviation standard the data were statistically not significant, while the treatment of yellow root brackish water decoction did not change the SGOT levels. Both the treatment of yellow root water decoction and brackish water decoction did not influence bilirubin levels. The interesting result of this research was in the correlation of SGPT levels and histopathological data of liver shown in Figure 1. Table 3 showed the data of SGPT levels and only the biggest dose of yellow root brackish water decoction increased SGPT level in comparison to normal control, while two other doses did not change the levels and their microscopically view did not show lipid degeneration or steatosis. The treatment of all doses of yellow root water decoction, increased statistically and clinically significant SGPT levels and interestingly they were in line with microscopically view of liver even only steatosis condition and there were no inflammatory infiltration nor necrosis. It must be noted that the SGPT levels of rats treated with yellow root water decoctions were very high, until 64.04, 59.36 and 68.12 U/L. They were significantly highest than normal range levels (16-48 U/L). It will be interesting if the length of treatment is not 28 days but extend until 90 days.

Even though it can be concluded that there were different influences between the treatment of yellow root water decoction and those of brackish water decoction. The use of brackish water decoction is safe and this is really an example of local wisdom because Dayak tribes in Borneo usually use the yellow root boiled with brackish water. This is half clarification of ethnomedicine. Is there any correlation with berberine levels contained in these two preparation methods?

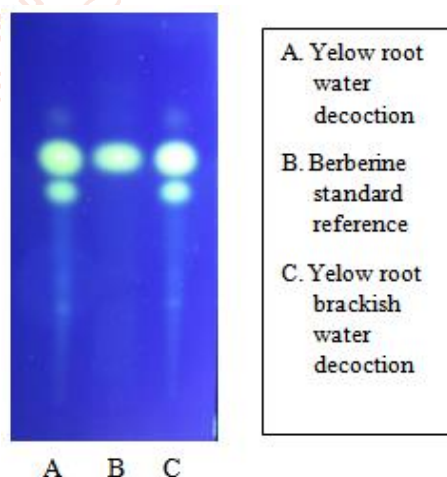


Figure 2. Silica gel 60 F₂₅₄ HPTLC profile of yellow root water and brackish water decoction with upper phase of n-butanol-acetic acid-water (4:1:5 v/v) as mobile phase, detected under UV₃₆₆.

The high performance thin layer chromatography (HPTLC) showed a dominant yellow fluorescence spot under UV₃₆₆ both in the yellow root water and brackish water decoction such as shown in figure 2. The determination of the berberine level using HPTLC-Densitometry at 450 nm showed that yellow root water decoction contained 0.99% berberine, while yellow root brackish water decoction

contained 1.38% level of berberine. It seems that there is contradictory between these berberine levels and the data of subchronic toxicity especially in liver damage. Was there reaction between berberine and chemical content of brackish water?

According to Zefrina, et al., (2015) technically, brackish water contains between 0.5 and 30 grams of salt per litre—more often expressed as 0.5 to 30 parts per thousand (‰), which is a specific gravity of between 1.005 and 1.010. Thus, brackish covers a range of salinity regimes and is not considered a precisely defined condition. It is characteristic of many brackish surface waters that their salinity can vary considerably over space or time. Referring to HPTLC profile of yellow root brackish water decoction in figure 2, it consisted exactly similar spots with that of yellow root water decoction. It means that there was no reaction between chemical content of brackish water and berberine. The other possibility is the influence of brackish water to the absorption of berberine in the intestine. It will be programmed in future research. Referring to 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, for substances containing berberine such as *Berberis* spp, *Coptis* spp, including *Arcangelisia flava*, the recommended warning statements are as follow: “Not to be taken by: babies, children under 12 years of age, pregnant women or lactating mothers.. Consult your practitioner if you have conditions such as Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency, haemolytic anemia, glaucoma, diabetes, high blood pressure, history of cardiovascular disease or if you are using paclitaxel, cyclosporin or other chemotherapeutic agents”. Three ASEAN member states still use substances containing berberine with restriction, three others depend on the national regulatory authority and four members including Indonesia and Malaysia do not allowed the use the substances containing berberine as component of Traditional Medicines and Health Supplements. Based on this research we recommend to apply the restriction in the label if yellow root is used in a commercial product. In the other side people can still use traditionally the brackish water decoction as local wisdom based on our ethnomedical research. 5.

5. Conclusion

Based on the results of the research and the discussion, it can be concluded that:

Yellow root water decoction had toxic effect in liver function while yellow root brackish water decoction did not cause toxic effect after 28 days treatment in female Wistar rats. The yellow root water decoction contained 0.99% berberine, while the yellow root brackish water decoction contained 1.38%. It was suggested the possibility of inhibition of berberine absorption by brackish water.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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