

Full Length Research Paper

Evaluation of hematological and biochemical activity of *Bauhinia strychnifolia* Craib leaves ethanol extracts in normal albino rats

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The aim of this research is to evaluate the hematological and biochemical activity of *Bauhinia strychnifolia* Craib leaf ethanol extracts in normal albino rats. Thirty-two male and female rats were divided into four groups, each with equal numbers of male (n=4), female (n=4). The control group was administered 1 ml of 10% tween 80. The experimental groups were administered ethanol with concentrations of 125, 250 and 500 mg/kg body weight (bw). After 21 days of treatment, the blood samples were collected for hematological: Red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), white blood cell (WBC), neutrophil (NEU), lymphocyte (LYMPH), monocyte (MONO), eosinophil (EO), basophil (BASO) and biochemical analysis; glucose (GLU), blood urea nitrogen (BUN), creatinine (CREA), cholesterol (CHOL), and triglyceride (TG). The results showed that both male and female rats that received 500 mg/kg bw Craib leaf ethanol extract have increased RBC, HGB, HCT and WBC. The hematological activity in male rats increased significantly ($p < 0.05$) compared to the control group. The biochemical activity in male rats administered with 125 mg/kg bw significantly ($p < 0.05$) increased BUN, CHOL, and TG compared to the control group. No evidence of abnormalities was observed.

Key words: *Bauhinia strychnifolia* Craib, hematological, biochemical.

INTRODUCTION

Bauhinia strychnifolia Craib belong to the Leguminosae-Caesalpinaceae family. The leaves and stems (Figure 1) have been used in Thai traditional medicine for treatment of fever and, alcohol intoxication (Wutthithammavet, 1997; Pichiansoonthon and Mhokkakul, 2001; Wuthithamaweeh, 2004) is frequently incorporated in herbal tonics that supplement blood circulation and bone functions (Thangthaisong et al., 2011). Preliminary phytochemical testing of ethanol extract *B. strychnifolia*

showed the presence of flavonoids, alkaloids, tannins, and cardiac glycosides (Kraithep et al., 2017; Laksungnern et al., 2019). All four types of phytochemicals have a large pharmacological effect, neurology covers many systems of the body and classified as natural pharmaceuticals that are used for medical benefits (Thangthaisong et al., 2011). Research studies have also indicated anticancer, anti-HIV, antimalaria, antioxidant, and cytotoxic properties of *B.*

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Figure 1. (a) Stem, (b) leaf and (c) flower of the *Bauhinia strychnifolia*.

strychnifolia leaves and stems (Laksungnern et al., 2019; Yuenyongsawad and Bunluepuech, 2013; Bunluepuech and Tewtrakul, 2011; Itharat et al., 2013; Panchinda et al., 2016).

The *in vitro* pharmacology activity of *B. strychnifolia* leaves and stems have shown many kinds of cells; however, information regarding their hematological and biochemical activity is yet to be reported. Therefore, this study aims to determine the hematological and biochemical activity of *B. strychnifolia* leaf ethanol extract in normal albino rats.

MATERIALS AND METHODS

Plant and extraction

The leaves of *B. strychnifolia* were collected from Thanyaburi, Pathumthani, Thailand and authenticated at the botany department of Forest herbarium, Bangkok, Thailand (specimen ID: BKF NO.195599). The leaves were air-dried at room temperature before they were ground and sieved to fine power. The fine powder (1.7 g) was macerated in 10 g of 95% ethanol. The macerated mixture was evaporated at 60°C in heat water bath (yield = 15.62% w/w). It was kept at -20°C throughout the experiment.

Experimental animal

Thirty-two Wistar strain rats, male (n=16) and female (n=16), with initial weight ranging between 200 and 250 g, were used in the experiments. All rats were housed at the Thai Traditional Medicine College, Rajamangala University of Technology Thanyaburi, Thailand, under standard environmental conditions (24 ± 1°C, 60 to

70% humidity, 12-h light: 12-h dark cycle). Food and water were given *ad libitum*. All experimental procedures were conducted according to the Animal Care and Use Committee guidelines, Rajamangala University of Technology Thanyaburi, Thailand (Animal License No.RMUTT.TMC.2018. R001).

Hematological and biochemical analysis

The rats were randomly divided into four groups, each with equal numbers of male (n=4) and female (n=4). The concentrations used in the experiment are based on previous toxicity experiments from Voravuth et al. (2015). The experiment was modified from Omoboyowa et al. (2016) and Haytham (2016). The rats were given *B. strychnifolia* leaf ethanol extract orally with concentrations of 125, 250, and 500 mg/kg/ body weight (bw), the control group was administered 1 ml of 10% tween 80 for 21 days. All animals were fasted for 8 h and sacrificed on day 21 with intraperitoneal injection of 150 mg/kg bw thiopental. Blood samples were collected by cardiac puncture into EDTA coated tube. The hematological and biochemical analysis was performed at the National Laboratory of Mahidol University to determine red blood cell (RBC) counts, hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), white blood cell (WBC), neutrophil (NEU), lymphocyte (LYMPH), monocyte (MONO), eosinophil (EO), basophil (BASO), glucose (GLU), blood urea nitrogen (BUN), creatinine (CREA), cholesterol (CHOL), and triglyceride (TG). Moreover, a necropsy exam was performed on the liver, spleen, heart, lung, ovary, and uterus.

Statistical analysis

The results were expressed as means ± SEM. Statistical difference between the means was determined by one-way analysis of variance (ANOVA), followed by Dunnett's tests which treated one

Table 1. Effect of *B. strychnifolia* leaf ethanol extract on hematological parameters of Wistar rats after 21 days oral administration.

Parameter	Treatments			
	Control	125 mg/kg bw	250 mg/kg bw	500 mg/kg bw
Male (n=4)				
RBC ($10^6/\mu\text{l}$)	7.78±0.21	7.58±1.84	7.84±0.17	8.21±0.11
HGB (g/dl)	14.28±0.36	14.25±0.33	14.10±0.33	14.63±0.40
HCT (%)	41.25±1.45	41.13±0.84	40.35±0.98	42.63±1.12
MCV (fl)	53.00±0.41	54.28±0.79	51.38±0.63	51.90±0.79
WBC ($\times 10^3\text{mm}^3$)	5.80±0.43	6.35±0.42	6.85±0.45	8.96±0.55*
NEU (%)	8.45±2.56	8.43±0.69	6.65±1.81	8.10±1.00
LYMPH (%)	84.78±2.29	85.98±0.69	89.03±2.23	88.08±1.41
MONO (%)	5.75±0.65	4.58±0.68	3.00±0.41*	2.93±0.47*
EO (%)	0.98±0.03	0.95±0.19	1.15±0.17	0.85±0.09
BASO (%)	0.05±0.05	0.08±0.05	0.18±0.08	0.08±0.03
Female (n=4)				
RBC ($10^6/\mu\text{l}$)	7.41±0.11	7.53±0.12	7.16±0.11	7.69±0.26
HGB (g/dl)	13.73±0.31	13.73±0.09	13.35±0.19	13.88±0.35
HCT (%)	39.83±1.11	39.48±0.21	38.60±0.38	40.55±1.19
MCV (fl)	53.70±0.71	52.48±0.83	53.98±0.35	52.80±0.93
WBC ($\times 10^3\text{mm}^3$)	4.46±0.26	4.56±0.16	5.13±0.53	5.36±0.54
NEU (%)	4.43±1.66	5.78±1.30	9.53±1.34	6.58±2.11
LYMPH (%)	83.83±1.57	90.80±0.99	86.53±0.98	89.38±1.99
MONO (%)	3.18±0.24	2.45±0.33	2.88±0.50	3.15±0.28
EO (%)	0.78±0.17	0.88±0.10	1.03±0.08	0.90±0.09
BASO (%)	0.00±0.00	0.10±0.06	0.05±0.05	0.00±0.00

Red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), white blood cell (WBC), neutrophil (NEU), lymphocyte (LYMPH), monocyte (MONO), eosinophil (EO), basophil (BASO). *Significant at $p < 0.05$.

group as a control and compare all other groups. The significant difference was observed at $p < 0.05$.

RESULTS

Hematological parameters from both male and female rats showed increased RBC, HGB, HCT, and WBC levels when the rats received 500 mg/kg bw *B. strychnifolia* leaf ethanol extract. The male rats treated with 500 mg/kg bw *B. strychnifolia* leaf ethanol extract showed significant ($p < 0.05$) increase in WBC (8.96 ± 0.55) compared to the control group (5.80 ± 0.43). Neutrophils, lymphocytes, and eosinophils increase in female rats (not significant), through the levels were found to decrease in male rats that received extract doses of 250 and 500 mg/kg bw ($p < 0.05$) as shown in Table 1.

The biochemical parameters after 21 days oral administration of *B. strychnifolia* leaf ethanol extract is shown in Table 2. The results showed male rats administered with 125 mg/kg bw significantly increased BUN, CHOL, and TG ($p < 0.05$) levels compared to the control group. The biochemical parameters in female rats

did not show significant differences at any dose. Necropsy results showed no abnormalities on the liver, spleen, heart, lungs, ovaries, and uterus. All animals are healthy and no illness.

DISCUSSION

Oral administration of *B. strychnifolia* leaf ethanol extract to Wistar rats showed significant increase of BUN, CHOL, TG ($p < 0.05$) levels in male rats that received the extract dose of 125 mg/kg bw. The biochemical parameters of female rats, on the other hand, did not exhibit any significant change at any extract dose. These parameters are considered normal when compared with the national laboratory reference ranges according to animal age and gender.

Hematology results showed that RBC, HB, and HCT increased in rats that received 500 mg/kg bw leaf extract. All three are components of the blood, with HB being the components in red blood cells responsible for binding oxygen in the lungs and transferring to various tissues. HCT is the value of blood density, responsible for

Table 2. Effect of *B. strychnifolia* leaf ethanol extract on biochemical parameters of Wistar rats after 21 days of oral administration

Parameter	Treatments			
	Control	125 mg/kg bw	250 mg/kg bw	500 mg/kg bw
Male (n=16)				
GLU	155.28±15.53	186.30±40.31	122.88±7.03	124.35±10.12
BUN	11.15±1.07	15.00±0.49*	11.53±0.89	12.35±0.72
CREA	0.26±0.02	0.28±0.02	0.22±0.02	0.30±0.01
CHOL	56.40±3.05	74.75±7.82*	59.93±2.29	68.98±1.92
TG	72.90±8.67	118.40±6.64*	83.45±12.83	76.88±13.83
Female (n=16)				
GLU	142.73±12.89	123.38±6.67	103.88±19.35	163.00±25.53
BUN	10.75±0.86	10.38±0.78	9.30±1.23	10.58±0.48
CREA	0.27±0.01	0.28±0.02	0.26±0.03	0.26±0.01
CHOL	60.33±7.57	59.98±5.35	49.10±3.37	56.98±4.11
TG	79.83±8.33	72.45±7.11	55.75±13.66	106.78±31.54

Glucose (GLU), blood urea nitrogen (BUN), creatinine (CREA), cholesterol (CHOL), triglyceride (TG).
*Significant at $p < 0.05$.

stimulating the kidneys to make erythropoietin, which is used in the production of red blood cells. The extract dose of 500 mg/kg bw, which increases RBC, HB, and HCT, may have the ability to add substances to the blood composition. *B. strychnifolia* leaves are one of the herbs used to nourish female blood, according to Thai traditional medicine formula. In future studies, herbs in other blood tonic formula will be tested for its effects on altering the blood composition, and whether the effects are similar to what was observed from *B. strychnifolia* leaves. Extract doses of 125, 250 and 500 mg/kg bw increased blood neutrophil, lymphocyte and eosinophil counts in female rats. Similarly, increased lymphocyte counts were observed in male rats, and also WBC, which increased significantly ($p < 0.05$). All three types of WBC are components of the immune system that protects the body. Neutrophils are the most abundant type of white blood cell that destroys, catch germs and foreign bodies through phagocytosis. Lymphocyte is a type of white blood cell that is second most abundant from neutrophil. It fights chronic bacterial infections and acute viral infections. For example, T lymphocytes (T cells) target virus-infected cells and tumor cells, while B lymphocytes (B cells) produce antibodies, to eliminate foreign objects, and eosinophils are responsible for allergic reaction against parasites such as flatworms or roundworms. Increased white blood cells are not the only indication for infection, because an infection that causes inflammation must incur other symptoms such as fever, pain, swelling, redness (Prinyakupt and Pluempitiwiriawej, 2015; Medzhitov and Janeway, 2000; Chen et al., 2018). All experimental animals were in good health, and preliminary results of animal carcasses showed no abnormalities and this trial corresponds to the study of women's blood tonics and tonics for blood circulation and

bone functions. These results can promote the ethanol extract from *B. strychnifolia* leaves which increases the blood composition and stimulates the immune system in rats. Further experiments must be conducted to look at the stimulation of white blood cells isolated from human blood *in vitro*.

Conclusion

B. strychnifolia is a medicinal plant used in the prevention and treatment of diseases of Thai traditional medical knowledge. There are reports on the experimental properties attributed to this plant, the extracts of *B. strychnifolia* were found to be antidote from pesticides, toxic agents, anticancer, and antioxidation. The results of hematological and biochemical studies on *B. strychnifolia* leaves extract showed that it can be used to support local herbs used to nourish women and further developed as a blood tonic herbal medicine used in traditional Thai medicine, yet there is no literature report on human studies. With an increase in interest in this plant, the focus should be in the application of *B. strychnifolia* in the field of pharmacology specifically in studies focused on the treatment of anemia and immune dysfunction.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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