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# THE POTENCY OF *BAJAKAH* KALALAWIT (*Uncaria gambir* (W.Hunter) Roxb.) STEM EXTRACT TO REDUCE LEVELS MALONDIALDEHYDE (MDA) IN WISTAR RAT

**Nadia Putri Cahyani[1*], Agnes Frethernety[2], Septi Handayani[3]**
[1]Department of Medical Education, Faculty of Medicine, Universitas Palangka Raya, Palangka Raya, Central Kalimantan, Indonesia
[2]Departement of Pharmacology and Therapy, Faculty of Medicine, Universitas Palangka Raya, Palangka Raya, Central Kalimantan, Indonesia
[3]Departement of Biochemistry, Faculty of Medicine, Universitas Palangka Raya, Palangka Raya, Central Kalimantan, Indonesia
*email: nadiacahyani352@gmail.com

## ABSTRAK

Diabetes melitus (DM) ditandai dengan peningkatan kadar glukosa darah (hiperglikemia). Hiperglikemia tidak terkendali dapat mempercepat pembentukan senyawa oksigen reaktif (ROS) dan radikal bebas. Peningkatan radikal bebas disertai gangguan pertahanan antioksidan endogen mengakibatkan keadaan stres oksidatif dan akhirnya akan menghasilkan malondialdehid (MDA). Tujuan dari penelitian ini adalah untuk melihat potensi ekstrak batang *Bajakah* Kalalawit (*Uncaria gambir* (W.Hunter) Roxb.) terhadap penurunan kadar Malondialdehid pada tikus Wistar. Metode penelitian ini *True Experimental Design* yang menggunakan *Posttest Control Group Design* dengan Tikus putih jantan (*Rattus norvegicus)* Galur Wistar. Hewan percobaan dibagi menjadi lima kelompok terdiri. Semua kelompok tikus diinduksi streptozotocin dengan dosis 45 mg/kgBB kecuali kelompok normal (KN). Pada kelompok perlakuan diberikan ekstrak batang *Bajakah* Kalalawit dengan tiga dosis yaitu 50 mg/kgBB (P1), 75 mg/kgBB (P2), dan 100 mg/kgBB (P3) selama 21 hari. Pada hari ke-22 diambil sampel serum darah dan diukur kadar dengan menggunakan spektrofotometer panjang gelombang 500-600 nm. Hasil penelitian ini menunjukkan ekstrak batang *Bajakah* Kalalawit (*Uncaria gambir* (W.Hunter) Roxb.) dapat menurunkan kadar MDA pada tikus yang diinduksi streptozotocin karena mengandung saponin, alkaloid, flavonoid, tannin, steroid, dan triterpenoid yang bekerja dengan mekanisme antioksidan dan antihiperglikemia. Kelompok perlakuan pemberian ekstrak batang *Bajakah* Kalalawit pada dosis 100 mg/KgBB merupakan dosis efektif dalam menurunkan kadar malondialdehid pada tikus wistar yang diinduksi STZ.
**Kata Kunci :** *Uncaria gambir* (W.Hunter) Roxb., Hiperglikemia., Malondialdehid

## ABSTRACT

Diabetes mellitus (DM) is characterized by increased blood glucose levels (hyperglycemia). Uncontrolled hyperglycemia can accelerate the formation of reactive oxygen compounds (ROS) and free radicals. Increased free radicals accompanied by disruption of endogenous antioxidant defenses result in a state of oxidative stress and will eventually produce malondialdehyde (MDA). The purpose of this study was to see the potential of the extract of the stem of *Bajakah* Kalalawit (*Uncaria gambir* (W.Hunter) Roxb.) to reduce levels of Malondialdehyde in Wistar rats. This research method is True Experimental Design using Posttest Control Group Design with male white rats (*Rattus norvegicus*) Wistar strain. The experimental animals were divided into five groups. All groups of rats were induced by streptozotocin at a dose of 45 mg/kg BW except the normal group (KN). The treatment group was given the extract of *Bajakah* Kalawit stems with three doses, namely 50 mg/kgBB (P1), 75 mg/kgBB (P2), and 100 mg/kgBB (P3) for 21 days. On the 22nd day, blood serum samples were taken and levels were measured using a spectrophotometer with a wavelength of 500-600 nm. The results of this study indicate that the stem extract of *Bajakah* Kalalawit (*Uncaria gambir* (W.Hunter) Roxb.) can reduce MDA levels

in streptozotocin-induced rats because it contains saponins, alkaloids, flavonoids, tannins, steroids, and triterpenoids which work with antioxidant and antihyperglycemic mechanisms. The treatment group was given the extract of *Bajakah* Kalalawit stems at a dose of 100 mg/Kg BW which was an effective dose in reducing malondialdehyde levels in STZ-induced wistar rats.

**Keywords:** *Uncaria gambir* (W.Hunter) Roxb., Hyperglycemia., Malondialdehyde

## INTRODUCTION

Diabetes melitus (DM) is metabolic disorder that happened when the pancreas unable to produce insulin and the body unable to use insulin effectively. The blood glucose levels are tightly regulated in the body. Generally, the blood glucose levels stucked between 70-100 mg/Dl. The high blood glucose levels (hyperglycemia) caused by people who never controlled that has a negative impact, it effected diabetes melitus disease.[1]

The DM patient that has the high blood glucose levels can trigger the exeсessive production of *Reactive Oxygen Species* (ROS). The formation of free radicals in the form of reaction oxygen or *Reactive Oxygen Species* (ROS) in the patient's hyperglycemia through several mechanism, there are; autixidation, glucose, protein glycation and activation of the polyol.[2] the imbalance between free radicals and endogeneous antioxidants, such *superoksida dismutase* (SOD), *catalase* (CAT) dan *glutathione peroksidase* (GPx) which resulted from the body can effected oxidative stress, which is characterized of increasing levels of Malondialdehid (MDA). Oxidative stress can increase lipid peroxidation, it can damage the cell membrane from cellular to organ level, such as, blood vessel damage, nerve, and other internal structures. The evolution of lipid peroxidation in DM patients due to cronic hyperglycemia leads to the formation of Malondialdehyde (MDA) which can be used as a marker of oxidative damage.[3]

*Bajakah* stem is the characteristic plant of Central Kalimantan which uses to treat all kinds of diseases, one of them is as a theraphy for DM patient. *Bajakah* or called *Bajakah* Kalalawit, in the Anshari's research said that *Bajakah* stem were proven contains phenolic compounds, flavonoids, tannins, and saponins.[4] The research of Kasmudin said that active plant compound from Putri Malu plant containing alkaloids compound, tannins, and flavonoids that have potential as antihyperglikemia and antioxidant,[5] tannins compound provide protection for free radical. Saponins can increase the use of glucose and reduce the process of gluconeogenesis. Some of these compounds found in *Bajakah* stem. Based on the statement, this research is investigated the potential of stem extract *Bajakah* Kalalawit (*Uncaria gambir* (W.Hunter) Roxb.) for the reduction of Malondialdhyde levels of Wistar stem that inducted by streptozotocin (STZ).

## TOOLS AND METHODS

The tools of research are mask, handscoon, headcap, rat cage, water container, wood's powder, spuit 1 ml, alcohol swab, glucose strip test, glucometer, pipette, black cloth, glass vessel, mortar, stamper, filter paper, funnel, *waterbath*, porcelain cup, scales, analytic scale, *rotary evaporator*, knife, EDTA tube, centrifuge, UV spectrometer, micropipette, refrigerator, cotton, scissors, Nasogastric Tube, scalpel, ballpoint, volumetric flask and vortex.

The tools for this research are *Bajakah* Kalalawit (*Uncaria gambir* (W.Hunter) Roxb.), etanol 96%, white blood of male mice Galur Wistar, *aquadest*, Streptozotocin (STZ), buffer sitrat, pH 4,5 sucrose, Na-CMC, TCA 100%, Na-Thio 1%, dan HCL 1 N.

### Research Project

This research is *True Experimental Design* that used *Posttest Control Group Design* method. This research was approved by the Research Ethics Committee of the Faculty of Medicine, Palangka Raya University No.64/UN24.9/LL/2022

### Etanol Extract 96% of *Bajakah* Kalalawit Stem

*Bajakah* Kalalawit taken in Marang, Central Kalimantan. The weight of *Bajakah* Kalalawit stem is 7,5 kg. Then, it processed 2,17 kg and processed used ethanol solution for about 96% in 3x24 hours. The results of the extract obtained are 85,73 gr. Then, the doses *Bajakah* Kalalawit stem extract was made with the doses of 50 mg/KgBW, 75 mg/KgBW, 100 mg/KgBW according to the body weight of each rat.

### Experimental Animal Treatment

This research used male white rats as experimental animal (*Rattus norvegicus)* Galur Wistar with the several criteria: male rat, 2-3 months old, body weights 150-250g, healthy condition. There were 25 Wistar rats used, divided into 5 groups consisting of Normal (KN), Negatif groups (K-), *Bajakah* Kalalawit divided into 3 doses of extract stem, there were 50 mg/kgBB (P1), 75 mg/kgBB (P2), 100 mg/kgBB (P3).

The experimental animals were acclimatized for 7 days, placed in the cage and covered with husks, the food of rat is fed pellets and drinking water. After acclimatization the experimental animals were weighed to measure initial body and initial blood glucose before being induced streptozotocin using a glucometer. Induction of streptozotocin (STZ) in the intraperitoneal area at a dose of 45 mg/KgBW to obtain type 2.[6] DM model. After negative group (K-) and treatment group (P1, P2, P3) have been induction by STZ, it got ad libitum food and drink and sucrose 20%. After 72 hours, the experimental animals that have been induction by streptozotocin, the blood glucose would measure to knowing the blood glucose of normal and treatment groups. The experimental animals in hiperglikemia have blood glucose more than ≥ 200 mg/dL.[7]

the distribution of 20% sucrose and *Bajakah* Kalalawit stem extract by sonde oral in 21 days after hyperglycemia was found in the treatment groups. The distribution of negative groups got 20% sucrose in 21 days. In the 22 days, blood was taken and MDA levels were checked by reading the absorbance using a spectrophotometer with a wavalenght of 500-600 nm.

**Data Analysis**

This research used SPSS program and used Kruskal Wallis test. Then, used Mann Whitney test to looking for the groups that has differences of $p< 0,05$ grade.

**RESULT**

***Bajakah* Kalalawit (*Uncaria gambir* (W.Hunter) Roxb.)**

*Bajakah* Kalalawit originally from Marang, Central Kalimantan that identification in Directorate of Scientific Collection Management BRIN (Badan Riset dan Inovasi Nasional) in Cibinong, the identification results are as follows:

Group : Rubiaceae
type : *Uncaria gambir* (W. Hunter) Roxb.

Based on the result of qualitative phytochemical screening *Bajakah* Kalalawit (*Uncaria gambir* (W.Hunter) Roxb.) contains saponins, alkaloids, flavonoids, tannins, steroids and triterpenoids. Meanwhile, the result of quantitative phytochemical screening for *Bajakah* Kalalawit (*Uncaria gambir* (W.Hunter) Roxb.) can be seen in table 1.

**Table 1** Quantitative phytochemical screening results of *Bajakah* Kalalawit

| No | Tested Compounds | Average Level±SD |
|---|---|---|
| 1 | Triterpenoids(mg/ml) | 375,133±1,155 |
| 2 | Flavonoids(mg/ml QE) | 261,417±1,283 |
| 3 | Saponins(%) | 51,860±0,670 |
| 4 | Alkaloids(%) | 37,770±0,285 |
| 5 | Steroids(mg/ml) | 18,359±0,078 |
| 6 | Tannins(mg/mL GAE) | 0,426±0,011 |

**Rat Blood Malondialdehyde Levels**

Investigation of blood MDA levels of male Wistar White Rats was carried out using the absorbance results which then produced *Tetraethoxy Propane* (TEP) standard curve to prepare a linear regression equation. MDA levels were then calculated in nmol/L units using linear regression procedure.

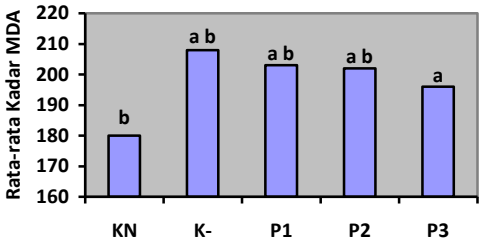
**Table 2** Rat Blood Malondehaldehyde levels (nmol/L)

| | Blood Malondehaldehyde levels (nmol/L) | | | | | Average Levels± SD |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | |
| KN | 181 | 181 | 180 | 181 | 180 | 180,6±0,547 |
| K- | 209 | 208 | 207 | 208 | 209 | 208,2±0,836 |
| P1 | 203 | 205 | 204 | 203 | 203 | 203,6±0,894 |
| P2 | 202 | 201 | 203 | 203 | 203 | 202,4±0,894 |
| P3 | 197 | 196 | 195 | 196 | 197 | 196,2±0,836 |

Informations:
KN : Normal Group
K- : Negatif Group(STZ)
P1 : Stem Extract *Bajakah* Kalalawit (STZ+dosis 50 mg/kgBB)
P2 : Stem Extract *Bajakah* Kalalawit (STZ+dosis 75 mg/kgBB)
P3 : Stem Extract *Bajakah* Kalalawit (STZ+dosis 100 mg/kgBB)

Based on Table 2 known that the average blood malondialdehyde (MDA) level of rat in the normal group was 180,6 nmol/L.Negstive group shown that the average malondialdehyde have been increase in 208,2 nmol/L. The treatment group of *Bajakah* Kalalawit have a decrease in rat malondialdehyde levels, for about 50 mg/kgBB dose was 203,6 nmol/L, a dose of 75 mg/kgBB was 202,4 nmol/L, and a dose of 100 mg/kgBB was 196 nmol/L.

The result of *Kruskal Wallis* test got the grade $p$=0,000 ($p$<0,05) showed statistically that there was a difference in *Bajakah* Kalalawit (*Uncaria gambir* (W.Hunter) Roxb.) stem extract in reducing MDA levels in Wistar rats induced by STZ. Statictic analysis of *Mann Whitney* test have the aim to find out the difference groups ($p$<0,05) can be seen in picture 1.




**Picture 1** *Mann Whitney* test for blood MDA levels

Informations:
a. : The difference significant in the Normal Groups
b. : The difference significant between the 100 mg/kgBB (P3) doses

Based on picture1 that the negative group (K-) and treatment group (P1, P2, P3) which induced streptozotocin combine glucose 20% shown that the levels of MDA increased than normal group (KN). MDA levels in normal group shown that the formation of free radicals is very smooth in Wistar rats without STZ, because in normal condition or the physiologic of free radicals are formed very slowly and still be reduced by endogenous antioxidants in the body. But, in negative group (K-) the MDA levels increased than normal group (KN). Moreover, the treatment group (P1, P2, P3) decreasly, it influenced by giving the *Bajakah* Kalalawit.

Based on *Mann Whitney* test in P3 group of *Bajakah* Kalalawit 100 mg/KgBB dose found that the difference than all of group (grade $p$>0,05). In the *Bajakah* Kalalawit 100 mg/kgBB shown that, the MDA levels decreased than *Bajakah* Kalalawit 50 dan 75 mg/kgBB in Wistar rat which induced by STZ.

**DISCUSSION**

The average result in MDA levels of in each group can be seen in table 2 that rat groups which induced by streptozotocin and combine with sucrose 20%. Then, the negative group (K-) without giving of and got the resulted that MDA levels increased than all of group, for about 208,2 nmol/L. Moreover, the rats in normal group (KN) have the lowest od MDA levels for about 180,6 nmol/L. The increased of MDA levels of negative (K-) group shown that the rat Wistar which induced by STZ with 40-55 mg/KgBB dose effected the partial insulin secretion disorder that resembles of types 2. The characterized of rat is the increased of fasting or intermittent blood glucose levels, decreased insulin levels and hyperglycemia. [6] It has the similarities of Imron R and friends research that analyzed the increased of blood glucose levels (hyperglycemia) and increased the MDA levels of rets which induced by STZ.[8]

The high glucose levels can trigger the formation of free radicals which result in lipid peroxidation leading to oxidative stress. When the free radicals exceeding the body's defense capacity, an imbalance occurs between free radicals and the body's antioxidants, such as superoxide dismutase, catalase, and glutathione peroxidase, causing oxidative stress.[10]

Hyperglycemia in DM can cause oxidative stress through several mechanisms, including autooxidation of glucose in the aldehyde group to form superoxide ($O_2^-$), hydroxyl radicals (OH*) and hydrogen peroxide ($H_2O_2$). The high glucose levels can increase the non-enzymatic glycosylation process in proteins caused by oxidation of the aldehyde groups in glocuse. This process caused the formation of *advanced glycation end products* (AGEs). Increased production of AGEs results in cell damage through 3 mechanism, such as, glucose autooxidation, methylglyoxal formation, and oxidative phosphorylation which ends in vascular damage so that blood MDA levels increase.[11] the binding of AGEs to macrophage receptors (RAGE) can lead to the synthesis of cytokines and growth factors as well as increased oxidative stress.[12] The oxidative stress causes execisive lipid peroxidation. The result of lipid peroxidation is MDA, so an increase of lipid peroxidation can cause an increase in MDA levels in the body.[13]

The decrease in average MDA levels of treatment group, namely Wistar rat that induced by STZ with the intervention of various doses of *Bajakah* Kalalawit, is influenced by the potency of *Bajakah* Kalalawit. The difference of doses in every treatment got difference respons in its network. The decrease of MDA levels caused by semipolar bioactive compounds in *Bajakah* Kalalawit. Based on phytochemical screening, the bioactive compounds contained in *Bajakah* Kalalawit were triterpenoids, flavonoids, saponins, alkaloidss, steroid, and tannins. Phytochemical compounds in plants have certain biological activities, such as, triterpenoids and flavonoids compound which proven to have acitivity in inhibiting the action of the alpha glucosidase enzim. Triterpenoids known that antidiabetic activity by stimulating and stabilizing the release of insulin from the β cells of the pancreatic islets of Langerhans, which have an alpha glucosidase enzyme inhibitory activity thereby preventing an increase in blood glucose.[14] based on Fariz and friends research, found that antioxidant in Flavonoid compounds that have OH groups. It act as antioxidant which safe progressive damage to pancreatic beta cells uccurs due to oxidative stress. Besides that, it can inhibit DNA methylation, the produce of NO and ROS combining with streptozotocin by releasing H ions. It caused DNA damaged, Hal ini mencegah terjadinya kerusakan DNA, the production of NO and ROS caused the flavonoids produksi NO dan produksi ROS sehingga flavonoid decrease in MDA levels. [15]

Based on the statistic analytic result in *Mann Whitney* test and picture 1 that the P3 groups (the doses of *Bajakah* Kalalawit 100 mg/kgBB) has a significant difference in reducing MDA levels compared to groups P1 (*Bajakah* Kalalawit dose 50 mg/KgBB) and P2 (*Bajakah* Kalalawit dose 75 mg/KgBB). The distribution of *Bajakah* Kalalawit in 100 mg/KgBB dose, it shown the effectivity to decrease MDA levels of Wistar rat which induced by STZ compared with *Bajakah* Kalalawit dose 50 and 75 mg/kgBB.

The lowest average grade of MDA in treatment group were 100 mg/kgBB for about 196,2 nmol/L difference with group for about 50 or 75 mg/kgBB. It happened as high as the dose, the highe effect got. The decrease of several dose produced the biggest respond. For the maximum effective dose, it placed all of receptor in the system. If the dose would give in the higher dose, it never high like maximum effect.[9] Based on several doses in this research, the effective dose in *Bajakah* Kalalawit has the potention to decrease MDA levels of rat which has inducted by STZ is 100 mg/kgBB.

The decrease in MDA levels of treatment groups difference than negative groups (Picture 1) indicated the STZ able increases glucose in the blood. The increased glucose levels trigger the formation of free radicals, resulting in lipid peroxidation which produces MDA. But, the active content of stem extraxt of *Bajakah* Kalalawit act as to inhibits the increased of MDA levels to improve hyperglycmia to inhibit the formation of free radical. This situation inhibit the peroxidation lipid which caused oxidative stress.[16] The stem of *Bajakah* Kalalawit content has antioxidant, such as; triterpenoids and flavonoids which have the greatest potential to reduce MDA levels. The toxic effect of *Bajakah* Tampala stem extract when used also need studied further to determine its safety limit when used.

**CONCLUSION**

Based on the research, the result is all of the *Bajakah* Kalalawit (*Uncaria gambir* (W.Hunter) Roxb.) stem extract able to reduced MDA levels in STZ induced for mouse. Then, from the three doses is the most effective to reduce MDA levels is 100 mg/KgBB doses.

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