

Effect of Sappanwood Extract (*Biancaea sappan* (L.) Tod.) on the Elimination Phase of the Pharmacokinetic Profile of Glibenclamide in Wistar Rats (*Rattus norvegicus*)

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ABSTRACT: Type 2 diabetes mellitus is characterized by chronic hyperglycemia, and glibenclamide remains a commonly used oral antidiabetic. Concomitant consumption of sappanwood extract (*Biancaea sappan* (L.) Tod.) is widespread in Indonesia, raising concerns about herb–drug interactions. This study investigated whether sappanwood modifies the elimination of glibenclamide in Wistar rats. Rats were randomized into three groups: Group I received glibenclamide 10 mg/kg BW; Group II received sappanwood extract 400 mg/kg BW; Group III received the combination. Blood was collected from the retro-orbital plexus at 20, 22, and 24 h and analyzed by UV-Vis spectrophotometry. Pharmacokinetic parameters were derived by linear regression and residual analysis. In Group I, the terminal log-linear profile yielded an elimination rate constant (K_e) of 0.1218 h^{-1} and an elimination half-life ($t_{1/2}$) of 5.79 h. In Group III, co-administration with sappanwood produced $K_e = 0.0066\text{ h}^{-1}$ and $t_{1/2} = 105\text{ h}$, indicating a pronounced slowing of glibenclamide elimination relative to control. Group II did not receive glibenclamide and was excluded from pharmacokinetic calculations. Sappanwood extract markedly altered glibenclamide disposition, consistent with a pharmacokinetic herb–drug interaction. The findings suggest that bioactive flavonoids (e.g., Brazilin and quercetin derivatives) may inhibit metabolic pathways relevant to glibenclamide clearance, leading to prolonged exposure.

Keywords: diabetes mellitus; elimination; glibenclamide; sappan wood (*Biancaea sappan* (L.) Tod.)

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1. Introduction

Diabetes Mellitus is a chronic metabolic disorder defined by persistent hyperglycemia due to impaired insulin secretion, insulin action, or both [1]. Type 2 diabetes represents approximately 90–95% of all cases and requires consistent glycemic control to prevent severe microvascular and macrovascular complications [2]. These conditions underline the need for therapeutic interventions that remain effective and safe over prolonged administration [3].

Glibenclamide, a commonly prescribed sulfonylurea, lowering blood glucose levels by stimulating insulin release from pancreatic β -cells [4]. However, the pharmacokinetics of glibenclamide can be affected by concomitant administration of other substances, including herbal products, which may modify its therapeutic efficacy [5]. With the increasing use of herbal remedies in the management of hyperglycemia, the evaluation of potential drug-herb interactions has become increasingly important [6]. Sappanwood (*Caesalpinia sappan L.*) is one of the herbal products of particular interest, traditionally consumed in Indonesia for its purported health-promoting effects.

Sappanwood (*Caesalpinia sappan L.*) exhibits a range of pharmacological properties, including antioxidant, anti-inflammatory, hepatoprotective, and antidiabetic activities. The primary bioactive compounds, such as brazilin and sappanchalcone, have been reported to modulate glucose metabolism and enhance insulin sensitivity [1]. Brazilin, a water-soluble flavonoid responsible for the extract characteristic red color, contributes to these pharmacological effects through its potent antioxidant activity [7]. Synergistic interactions between brazilin and other phytochemical constituents have been demonstrated, providing additional evidence for the therapeutic potential of sappanwood [8]. Additionally, sappanwood extracts exhibit inhibitory activity against α -amylase and α -glucosidase, enzymes involved in carbohydrate digestion [9]. Furthermore, bra-

zilin has exhibited dipeptidyl peptidase-IV inhibitory activity, thereby enhancing incretin function [10]. Holidah *et al.* demonstrated that Sappanwood extract exerted significant hepatoprotective and antioxidant effects in alloxan-induced diabetic rats, with the 400 mg/kg BW dose producing the lowest malondialdehyde (MDA) levels, indicating a strong potential to reduce oxidative stress [11].

Given the widespread use of glibenclamide and the increasing popularity of sappanwood as a complementary therapy, it is crucial to understand their potential pharmacokinetic interactions. Modifications in glibenclamide elimination kinetics, specifically the elimination rate constant (K_e) and elimination half-life ($t_{1/2}$) may influence drug exposure and therapeutic duration [12]. This study controbutor to see the effect of sappanwood extract on the elimination phase of glibenclamide in Wistar rats by examining changes in its K_e and $t_{1/2}$ values.

2. Materials and methods

2.1. Plant material and extract preparation

Sappanwood (*Biancaea sappan (L.) Tod.*, synonym *Caesalpinia sappan L.*) used in this study was authenticated at the Laboratory of Pengujian UPF Pelayanan Kesehatan Tradisional, Dr. Sardjito General Hospital, under determination certificate number TL.02.04/D.XI.6/2772.241/2024. Authenticated confirmed that the sappanwood was the plant part selected for extraction.

A total of 700 g of dried powdered simplicia was extracted by maceration. The powder was immersed in 7 L of 96% ethanol (ratio 1:10 b/v) in a tightly closed container and maintained for 72 hours with occasional stirring while protected from sunlight. Following filtration process, the residue was re-macerated using 3.5 L of 96% ethanol (ratio 1:5 b/v) for an additional 48 hours. The combined filtrates were concentrated using a rotary evaporator at 40°C until a thick extract was obtained. The resulting viscous extract was

weighed 91.25 g. Extract yield was calculated as the weight of the thick extract relative to the initial simplicia weight, resulting in a yield of 13.03%. The extract was stored in a tightly sealed container at room temperature until further use.

2.2. Animal subject

Male Wistar rats, aged 2–3 months and weighing 200–300 g, were used in this study, as verified by certificate number 363/SMMF/I/2023. Ethical approval was obtained from STIKES Telogorejo Semarang under approval number 094/IV/KE/STIKES/2024.

A total of 24 rats were allocated into three groups, each group consisting of eight animals. The sample size was determined using the Federer approach to ensure sufficient statistical power for detecting treatment differences. Accordingly, eight animals per group satisfied the minimum experimental requirements.

Animals were acclimatized under standard laboratory conditions and fasted for 8 hours before treatment while still receiving water. The treatment groups were as follows: Group I: glibenclamide 10 mg/kg BW; Group II: sappanwood extract 400 mg/kg BW; Group III: combination of glibenclamide 10 mg/kg BW and sappanwood extract 400 mg/kg BW.

Blood collection was performed through the retro-orbital plexus. Following blood collection, EDTA was added as an anticoagulant, and trichloroacetic acid was added to precipitate plasma

proteins. Samples were centrifuged at 2500 rpm for 15 minutes to obtain the supernatant, which was then mixed with pro-analysis methanol before spectrophotometric analysis.

2.3. Dosage

Glibenclamide was administered orally at a dose of 10 mg/kgBW. Sappanwood extract was administered at a dose of 400 mg/kgBW. The 400 mg/kgBW dose was selected based on previous *in vivo* studies that reported significant biological activity at this concentration, including improvements in oxidative stress parameters and hepatoprotective effects. This dose was therefore considered effective and appropriate for evaluating potential interactions with glibenclamide in the present pharmacokinetic study. The final administration volume was prepared at a concentration of 1.25 mg/mL and administered orally using an oral gavage.

2.4. Determination of glibenclamide maximum wavelength (λ_{max})

A stock solution of glibenclamide was prepared at a concentration of 1000 ppm. From this stock, a series of standard solutions were prepared at concentrations of 32, 48, 64, 80, and 96 ppm. Each solution was scanned using a UV-Vis spectrophotometer within the appropriate wavelength range to determine the maximum absorbance. The wavelength that produced the highest absorbance was selected as the maximum wave-

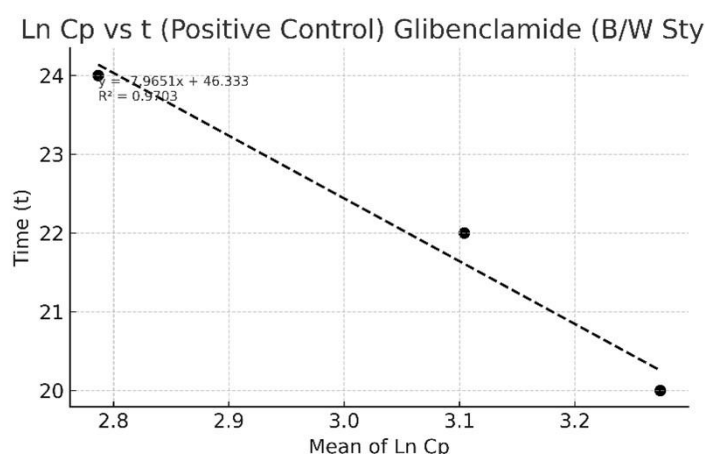


Figure 1. Ln Cp against t (positive control) Glibenclamide

length ($\lambda_{\max}$), which in this study was found to be 300 nm. The absorbance values of the standard solutions at 300 nm were then used to construct a calibration curve, and a linear regression equation ($y=bx+a$) was generated for subsequent quantitative analysis of glibenclamide in plasma samples.

2.5. Sampling preparation

The prepared doses of glibenclamide and sappanwood extract were administered orally using a 3 mL oral gavage. Blood samples were collected from the retro-orbital plexus at predetermined time points: 0, 1, 2, 4, 6, 8, 12, 20, 22, and 24 hours after administration. Approximately 500 μ L of blood was collected at each sampling point using a capillary tube.

Each blood sample was transferred into a centrifuge tube containing 500 μ L of 10% EDTA solution and 1000 μ L of 10% TCA solution to prevent coagulation and precipitate plasma proteins. The mixture was centrifuged at 2500 rpm for 15 minutes to obtain the clear supernatant. The resulting supernatant was then mixed with 2 mL of pro-analysis methanol, homogenized, and prepared for absorbance measurement using a spectrophotometer.

For pharmacokinetic analysis, only the terminal sampling points (20, 22, and 24 hours) were used to construct the elimination phase curve, as these time points provided quantifiable concen-

trations suitable for determining the elimination rate constant (K_e) and half-life ($t_{1/2}$).

2.6. Determining the pharmacokinetic profile

The residual method was useful to determine the pharmacokinetic profile of glibenclamide by analyzing the drug concentration data in the blood obtained at each time point and creating the average plasma concentration curve $\ln C_p$ versus time (t). The y-axis represented plasma concentration, while the x-axis represented time. The study analyzed the pharmacokinetic profile, specifically the elimination half-life ($t_{1/2}$) and elimination (K_e), using Excel software.

3. Results and discussion

This study evaluated the pharmacokinetic parameters of glibenclamide using Wistar rats (*Rattus norvegicus*) as the experimental subjects. Group I received glibenclamide 10 mg/kg BW, Group II received sappanwood extract 400 mg/kg BW, and Group III received the combination of glibenclamide 10 mg/kg BW and sappanwood extract 400 mg/kg BW. In the glibenclamide-only group (Group I), the elimination rate constant (K_e) was 0.1218/hour with an elimination half-life ($t_{1/2}$) of 5.79 hours, which is consistent with the expected elimination profile of glibenclamide. In contrast, the combination group (Group III) showed a markedly reduced elimination rate

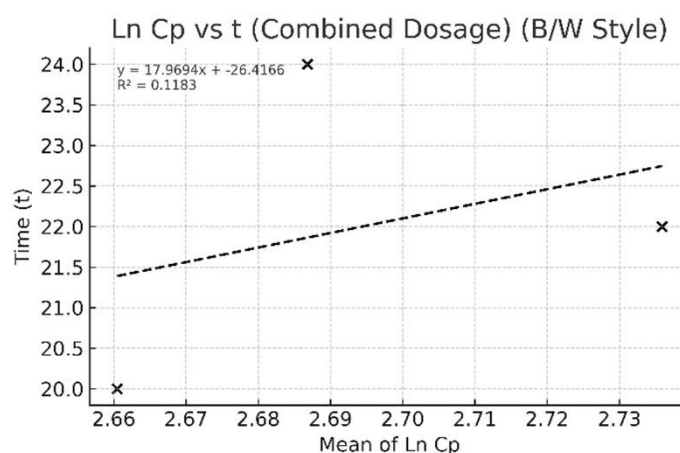


Figure 2. Ln Cp against t of combined dosage

constant of 0.0066/hour and a notably prolonged half-life of 105 hours. The decrease in K_e was associated with a pronounced increase in $t_{1/2}$, consistent with the pharmacokinetic principle that a lower elimination rate results in a longer elimination half-life. These finding indicates that co-administration of sappanwood extract may slow the clearance of glibenclamide, potentially prolonging its duration of action in the body [13].

The elimination phase of glibenclamide was evaluated using plasma concentration data collected at 20, 22, and 24 hours. Figure 1 illustrates the *time (t) versus ln Cp* relationship for the glibenclamide-only group (Group I), based on the last three blood sampling time points. The linear regression was $t = -2.9651(\ln Cp) + 46.333$ with $R^2 = 0.9703$, indicating a strong fit consistent with terminal elimination. Because Figure 1 is plotted with time on the yaxis and $\ln Cp$ on the xaxis (inverse of the conventional presentation), the elimination parameters were calculated from the same dataset analyzed in the standard *ln Cp versus time* orientation, yielding an elimination rate constant ($K_e = 0.1218 \text{ hour}^{-1}$) and an elimination half-life ($t_{1/2} = 5.79$ hours). In Group III (glibenclamide 10 mg/kg BW + sappanwood extract 400 mg/kg BW), the elimination phase was assessed using the same terminal sampling time points, as shown in Figure 2. The regression was $t = 0.0066(\ln Cp) + 2.5495$ with $R^2 = 0.1183$; the slightly positive slope and low R^2 indicate that the data did not follow a clear log-linear decline, and the resulting estimates ($K_e = 0.0066 \text{ hour}^{-1}$; $t_{1/2} = 105$ hours) should therefore be interpreted with caution. Previous pharmacokinetic studies in nondiabetic human subjects have reported a comparable half-life of ~5.25 hours, supporting the expected elimination profile of glibenclamide under normal conditions [16].

The findings of this study indicate that sappanwood extract can alter the elimination parameters of glibenclamide, as reflected by changes in both K_e and $t_{1/2}$. When compared with the glibenclamide-only group (Group I; $K_e = 0.1218 \text{ hour}^{-1}$, $t_{1/2} = 5.79$ hours), the marked reduction in K_e

and prolongation of $t_{1/2}$ in Group III suggest an interaction affecting glibenclamide elimination. These observations are aligned with previous pharmacokinetic reports in which co-administered substances or formulation changes modify glibenclamide exposure, evidenced by shifts in AUC, C_{max} , and T_{max} that ultimately reflect altered absorption and/or clearance [14]. Prior studies also show that glibenclamide disposition is sensitive to modulation of hepatic cytochrome P450 activity, particularly CYP2C9, CYP2C11, and CYP3A2, which play key roles in its metabolic clearance; alterations in these pathways can change systemic exposure and elimination behavior, supporting the plausibility of the pattern observed here [15].

Figure 2 shows the determination of the elimination for the combined dosage (Group III: glibenclamide 10 mg/kg BW + sappanwood extract 400 mg/kg BW), based on the last three time points of blood sampling at 20, 22, and 24 hours. The obtained linear regression equation is $t = 0.0066(\ln Cp) + 2.5495$ with $R^2 = 0.1183$. This regression was used to calculate the elimination of glibenclamide. The results of the glibenclamide elimination parameter calculation yielded an elimination rate constant (K_e) of 0.0066 hour^{-1} and a half-life ($t_{1/2}$) of 105 hours.

The results of this study are consistent with existing theoretical concepts. Sappanwood contains a variety of natural flavonoid compounds, including brazilin, caesalpiniafenol A-D, quercetin-3,7-di-O-methyl ether, 3-deoxysappanone B, sappanone A, 3'-deoxy-4-O-methylepisappanol, 10,11-dihydroxydracaenone C, quercetin-3',4'-di-O-methyl ether, and 3'-deoxysappanone that contribute to its biological activity. Compounds such as quercetin-3,7-di-O-methyl ether and quercetin-3',4'-di-O-methyl ether are methylated derivatives of quercetin, a flavonoid well known for its antidiabetic properties [17]. Quercetin is a polyphenolic flavonoid widely recognized for its antidiabetic activity, exerting effects such as reducing intestinal glucose absorption, enhancing insulin secretion, improving insulin sensitivity,

and promoting glucose uptake in muscle tissues. In human plasma, quercetin is predominantly present in glucuronidated, sulfated, and methylated forms, reflecting extensive hepatic phase II metabolism. Its multiple hydroxyl groups contribute to its strong antioxidant properties and structural reactivity, which underlie many of its biological effects [18]. Beyond its glucose-regulating effects, quercetin and structurally related flavonoids are also recognized for their modulatory influence on hepatic biotransformation pathways, which may alter drug disposition, enzyme affinity, and metabolic turnover rates [19]. This further supports the potential for sappanwood constituents to interfere with glibenclamide metabolism.

The observed differences in the elimination rate constant and half-life between Group I (glibenclamide alone) and Group III (combination) likely reflect interactions between glibenclamide and the phytochemical constituents of sappanwood extract. Previous findings indicate that herbal compounds can modulate hepatic CYP2C9 activity, providing a mechanistic basis for potential alterations in glibenclamide metabolism. [20]. Quercetin, one of the major flavonoids present in sappanwood, is known to inhibit several cytochrome P450 isoenzymes, including CYP2C8, CYP2C9, and CYP2C19. Its free hydroxyl groups enable binding interactions with these enzymes, potentially altering drug metabolism. The observed differences in elimination rate constant and half-life across treatment groups likely reflect interactions between glibenclamide and the phytochemical constituents of sappanwood extract. Glibenclamide is primarily metabolized in the liver by cytochrome P450 enzymes, particularly CYP2C9 and, to a lesser extent, CYP3A4, as demonstrated in previous pharmacokinetic studies. Variation in the activity of these metabolic pathways can alter the rate of glibenclamide biotransformation and thereby influence its elimination profile. Flavonoids such as quercetin, which are present in sappanwood extract, undergo extensive enzymatic processing and are known to in-

teract with hepatic drug-metabolizing systems. Evidence shows that quercetin exerts concentration-dependent inhibitory effects on cytochrome P450 enzymes, especially CYP3A, and suppresses P-glycoprotein expression, indicating its capacity to modulate both metabolic and transport processes. Such inhibitory actions provide a plausible mechanistic explanation for the reduced elimination and prolonged half-life of glibenclamide observed in this study [19].

These findings align with the pharmacokinetic principle that a decrease in the elimination rate results in a corresponding increase in elimination half-life ($t_{1/2}$), indicating a longer period required for the body to eliminate 50% of the drug [21]. The calculation of the elimination half-life ($t_{1/2}$) indicates that administration of sappanwood extract influences glibenclamide elimination profile. This is reflected by the decrease in the elimination rate constant (K_e) accompanied by a corresponding increase in $t_{1/2}$, demonstrating a slower elimination process.

4. Conclusions

Co-administration of sappanwood extract (400 mg/kg BW) with glibenclamide (10 mg/kg BW) produced a marked alteration in the pharmacokinetic elimination of glibenclamide in Wistar rats. The combination group demonstrated a substantial reduction in the elimination rate constant ($K_e = 0.0066 \text{ h}^{-1}$) and a pronounced prolongation of the elimination half-life ($t_{1/2} = 105 \text{ h}$) compared with the glibenclamide-only group ($K_e = 0.1218 \text{ h}^{-1}$; $t_{1/2} = 5.79 \text{ h}$). These findings indicate that sappanwood extract may slow the metabolic clearance of glibenclamide, leading to extended systemic exposure. The observed interaction is likely mediated by the inhibitory effects of sappanwood flavonoids, particularly quercetin derivatives, on hepatic cytochrome P450 enzymes involved in glibenclamide metabolism. Overall, the results suggest a potential herb-drug interaction that warrants careful consideration

when sappanwood preparations are co-administered with glibenclamide.

5. Limitation

This study has several limitations. The pharmacokinetic analysis was based on a limited number of sampling points in the elimination phase, which may influence the precision of the calculated elimination parameters. In Group III, the positive slope and low R^2 challenge the log-linear assumption, so the 105h $t_{1/2}$ should be viewed as an approximate indicator of slower elimination rather than a precise estimate. In addition, the study did not directly measure metabolic enzyme activity, so the proposed interaction mechanism remains theoretical. Further research with denser terminal sampling and biochemical assessments (e.g., CYP activity and transporter evaluation) is warranted to confirm these findings and clarify the clinical implications, including the possibility that sappanwood preparations could prolong glibenclamide exposure.

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