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PROFILE OF CYTOTOXICITY IN HepG2 CELLS AND THE POTENTIAL FOR MODULATION OF PANCREATIC CELLS BY PINEAPPLE CROWN FRACTION (*Ananas comosus* (L.)Merr) IN DIABETIC RATS

Profil Sitotoksisitas pada Sel HepG2 serta Potensi Fraksi Mahkota Nanas (*Ananas comosus* (L.) Merr.) dalam Memodulasi Sel Pankreas pada Tikus Diabetes

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ABSTRACT

The ethyl acetate fraction of pineapple crown, *Ananas comosus* (L.) Merr is known to contain flavonoid secondary metabolites with potential antidiabetic activity. Flavonoids have been widely reported to have potent antidiabetic effects through antioxidant activity and pancreatic cell-protective mechanisms. This study aimed to evaluate the in vitro toxicity profile of the ethyl acetate fraction using HepG2 cells and its in vivo antidiabetic efficacy in an alloxan-induced diabetic rat model. Cytotoxicity was assessed using the MTT assay, with Doxorubicin HCl used as a positive control. The in vivo study involved 35 male Wistar rats divided into seven groups: a negative control, a positive control (glibenclamide 0.45 mg/kgBW), and five treatment groups receiving the ethyl acetate fraction at doses ranging from 10 to 25 mg/kgBW. The evaluated parameters included changes in body weight, blood glucose levels, and pancreatic α- and β-cell counts. The cytotoxicity assay showed that the ethyl acetate fraction exhibited an IC_{50} value of 287.14 µg/ml, indicating relatively low toxicity compared with Doxorubicin HCl (IC_{50} =43.25 µg/ml). In vivo administration of the ethyl acetate fraction, particularly at a dose of 22 mg/kgBW, produced the most optimal effects, as indicated by body weight recovery toward normal values, a significant reduction in blood glucose levels, a decrease in pancreatic α-cell count (40±1.7), and an increase in β-cell number (48 ± 1.7) ($p < 0.05$). The ethyl acetate fraction of *Ananas comosus* (L.) Merr demonstrates antidiabetic potential by improving islet cell profiles while exhibiting moderate cytotoxicity in vitro, suggesting its potential for further development as an antidiabetic therapeutic agent.

Keywords: *Antidiabetic, α-cells, Cytotoxicity, Ethyl acetate fraction, HepG2*

ABSTRAK

Fraksi etil asetat mahkota nanas (*Ananas comosus* (L.) Merr.) diketahui mengandung metabolit sekunder flavonoid yang berpotensi sebagai antidiabetes. Flavonoid telah banyak dilaporkan memiliki efek antidiabetes melalui aktivitas antioksidan dan mekanisme perlindungan sel pankreas. Penelitian ini bertujuan untuk mengevaluasi profil toksisitas *in vitro* fraksi etil asetat menggunakan sel HepG2 serta efektivitas antidiabetes *in vivo* pada model tikus diabetes yang diinduksi aloksan. Uji sitotoksisitas dilakukan menggunakan metode MTT assay dengan doksorubisin HCl sebagai kontrol positif. Penelitian in vivo menggunakan 35 ekor tikus jantan Wistar yang dibagi menjadi tujuh kelompok, yaitu kontrol negatif, kontrol positif (glibenklamide 0,45 mg/kgBB) dan lima kelompok perlakuan yang menerima fraksi etil asetat pada dosis 10 hingga 25 mg/kgBB. Parameter yang diamati meliputi perubahan berat badan, kadar glukosa darah, dan jumlah sel α dan β pankreas. Hasil uji sitotoksisitas menunjukkan bahwa fraksi etil asetat memiliki nilai IC50 sebesar 287.14 µg/ml yang mengindikasikan toksisitas rendah dibandingkan dengan doksorubicin HCl (IC_{50} = 43,25 µg/ml). Pemberian fraksi etil asetat

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secara in vivo, khususnya pada dosis 22 mg/kgBB, memberikan efek paling optimal yang ditunjukkan oleh pemulihan berat badan mendekati normal, penurunan kadar glukosa darah yang signifikan, serta penurunan jumlah sel α pankreas (40 ± 1,7) serta peningkatan jumlah sel β (48 ± 1,7) ($p < 0,05$). Fraksi etil asetat menunjukkan potensi antidiabetes melalui perbaikan kerusakan sel pulau Langerhans dengan tetap menunjukkan sitotoksisitas sedang secara in vitro, sehingga berpotensi untuk dikembangkan lebih lanjut sebagai agen terapeutik antidiabetes.

Kata Kunci: *Antidiabetes, Sel α, Sitotoksisitas, Fraksi Etil asetat, HepG2*

INTRODUCTION

Diabetes mellitus has become one of the most frequently discussed diseases in various studies (Liu et al. 2022). It is a chronic condition characterized by persistent high blood sugar levels due to impaired insulin function, either in terms insulin production or efficiency insulin sensitivity, with ultimately affects the metabolism of carbohydrates, lipids, and proteins in the body (Shobana et al. 2009). Effective management of diabetes requires regular monitoring of blood glucose levels, along with necessary dietary and activity changes, and pharmacological therapy, including insulin or oral hypoglycemic agents (Tanko et al. 2008).

Glibenclamide (Glyburide) is an antidiabetic drug belonging to the sulfonylurea class, which is closely related to sulfonamide antibiotics (Edagha et al. 2021). Glibenclamide acts by binding to ATP-sensitive potassium channels in pancreatic β cells. This binding increases intracellular calcium levels in the β cells, thereby stimulating insulin secretion (WAHYUNI et al. 2024). However, despite their effectiveness in treating diabetes, some sulfonylurea drugs are associated with an increased risk of CVD -related mortality.

Consequently, Herbal products are increasingly explored as alternative antidiabetic therapies due to their perceived lower toxicity and potential multitarget effects. Pineapple (*Ananas comosus (L.)* Merr), a major agricultural commodity in Kediri, particularly in the Wates area, has been reported to contain high levels of phenolic compounds. Phenolic constituents, including flavonoids, have demonstrated considerable antidiabetic potential. Previous studies have identified several bioactive secondary metabolites in the ethyl acetate fraction of pineapple crown, including catechin and apiteginin, as well as amino acids such as isoleucine and arginine. The ethyl acetate fraction at 150 µg/mL has been shown to inhibit both α-glucosidase and α-amylase by ≥50% *in vitro* (- et al. 2024)

However, in vitro findings alone are insufficient to support the development of this fraction as a phytopharmaceutical agent, as comprehensive in vivo efficacy and cytotoxicity evaluations are required to ensure both therapeutic effectiveness and safety (Breban-Schwarzkopf et al. 2024). In this context, cytotoxicity assessment using HepG2 cells is commonly employed as a preliminary screening model to evaluate the general cellular toxicity of bioactive compounds in mammalian systems. Although HepG2 cells are derived from hepatocellular carcinoma and primarily represent hepatic responses, this model provides important initial safety information prior to in vivo application. It is important to note that such in vitro cytotoxicity evaluation does not directly reflect pancreatic-specific mechanisms but serves as an early indicator of potential toxicity.

In addition to α-cell alterations, pancreatic β-cell dysfunction plays a central role in the pathophysiology of diabetes mellitus. β-cells are responsible for insulin secretion, and their damage or loss leads to impaired glucose regulation and persistent hyperglycemia. Therefore, evaluating β-cell condition alongside α-cell modulation is essential to obtain a more comprehensive understanding of pancreatic endocrine responses. Several plant-derived compounds, particularly flavonoids, have been reported to protect β-cells by reducing oxidative stress and promoting cellular recovery (Almuttairi 2023; Dusaulcy et al. 2016).

Therefore, this study aimed to evaluate the antidiabetic efficacy and preliminary safety profile of the ethyl acetate fraction of pineapple crown at concentrations ≥150 µg/mL, through cytotoxicity testing (HepG2 cell viability), as well as in vivo assessment, including blood glucose levels and pancreatic α-cell counts in a diabetic rat model.

MATERIAL AND METHODS

Place and Time

The research was conducted at the Pharmacology laboratory, Faculty of Health Sciences, Kadiri University and the INVILAB laboratory from July to October 2025.

Material

The materials used in this study included 96% ethanol (Brataco, Cikarang, Indonesia), alloxan (Sigma Aldrich, Darmstadt, Germany), xylene (Merck, Darmstadt, Germany), hematoxylin-eosin (Leica, USA), paraffin (KunLun, China), ethyl acetate, chloroform (Brataco, Cikarang, Indonesia), pineapple crown, HepG2 cell, and aquadest.

Methods

Fractionation and Structural Characterization of Pineapple Crown Fraction

The ethanol extract of the pineapple crown was dissolved in an ethanol-water mixture and subsequently partitioned with n-hexane. The ethanol-water phase was collected and further partitioned using ethyl acetate. The resulting ethyl acetate fraction was evaporated under reduced pressure to yield a thick extract (winartiana et al. 2024) This ethyl acetate fraction was used for both in vitro and in vivo experiments.

In Vitro Toxicity Assessment

The in vitro cytotoxicity assay was conducted as a preliminary evaluation of the tested fraction's general cellular toxicity and was not intended to assess pancreatic-specific mechanisms.

Cell Culture

HepG2 human liver cancer cells were obtained from the INVILAB facility and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. Cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 24 hours prior to treatment (Sakao et al. 2023).

MTT Assay for Cell Viability

Cell viability was assessed using the MTT assay according to established protocols (Sakao et al. 2023). HepG2 cells (9.35×104 cells/well) were seeded into 96-well plates. Cells were treated with 50 µL of the ethyl acetate fraction at concentrations of 10, 25, 50, 100, and 200 µg/mL for 24 hours. Subsequently, MTT solution was added and incubated for 4 hours. The resulting MTT–formazan crystals were dissolved in 150 µL of DMSO, and absorbance was measured at 492nm using a Multiskan TMFC device (Thermo Scientific TM). Cell viability was calculated as the ratio of optical density between the treated and control groups. All experiments were performed in triplicate (technical replicates) and repeated three times (biological replicates).

In vivo Antidiabetic Evaluation (Rat Model)

The in vivo study was conducted to evaluate the antidiabetic efficacy and pancreatic effects of the fraction under physiological conditions.

Experimental Animals and Induction of Diabetes

Thirty-five male Wistar rats aged 8–10 weeks,180–250 g) were used in this study. Animals were acclimatized for 7 days prior to experimentation. All experimental procedures were approved by the institutional Animal Ethics Committee of Universitas Airlangga Faculty of Dental Medicine Health Research Ethical Clearance (Approval No: 0801/HRECC.FODM/VIII/2025).

The rats were randomly assigned to 7 groups (n= 5 per group) using a simple randomization method. Negative control (normal), Positive control (High Fat Diet (HFD) + alloxan + glibenclamide). Treatment group (HFD + alloxan + fraction doses of 10,15,18,22, and 25 mg/kgBW). Except for the negative control, all groups were fed a high-fat diet for 4 weeks. Diabetes was induced using a low dose of alloxan (35-40 mg/kgBW, intraperitoneally), followed by

administration of sucrose solution for 3 days to prevent acute hypoglycemia. The combination of a high-fat diet and low-dose alloxan was used to mimic insulin resistance and partial pancreatic β-cell dysfunction, representing a model of type 2 diabetes. Rats with fasting blood glucose (FBG) ≥ 126 mg/dl were considered diabetic (Fitriyanto et al. 2020)

Treatment Protocol

Rats in the positive and treatment groups were given daily treatment for 10 days. The positive control group received 0.45 mg/kgBW of glibenclamide, while treatment groups received ethyl acetate fraction at doses of 10, 15, 18, 22, and 25 mg/kgBW, respectively. All treatments were administered orally via gastric gavage. Fasting blood glucose levels were measured before and after treatment. At the end of the experiment (day 14), animals were euthanized for pancreatic tissue analysis.

Histopathological Examination of Pancreas

Pancreatic tissues were fixed in formalin and dehydrated in graded alcohols (70%, 80%, 90%, 95%, and 100%) for 30 minutes each. Tissues were cleaned in xylene for 1.5 hours until the xylene colour faded, and then cleaned with a mixture of xylene and paraffin substitute (ratios 3:1, 1:1, and 1:3 v/v) for 30 minutes. Subsequently, the tissues were infiltrated with molten paraffin at 58-60 °C for two cycles (1 hour each) and embedded in paraffin blocks. Tissue sections were stained using hematoxylin-eosin and examined under a light microscope at 400× magnification (Widyatmaka and Ismail 2021). Histopathological evaluation was performed by an independent observer blinded to group allocation.

Data Analysis

Data were expressed as mean ± standard deviation (SD). Normality of the data was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using Levene's test. For datasets that met the assumptions of normality and homogeneity, statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. For datasets that did not meet the normality assumption, nonparametric analyses were performed using the Kruskal–Wallis test followed by the Mann–Whitney U test with Bonferroni correction. A p-value < 0.05 was considered statistically significant. (Widyatmaka and Ismail 2021)

RESULT AND DISCUSSION

Phytochemical Composition of Ethyl Acetate Fraction

A total of 5.5 kg of pineapple crowns yielded 700 g of dried simplicial after being oven at 50 °C, with a moisture content of 7.6%, indicating adequate dryness for storage. Moisture content below 10% is generally considered optimal for preventing microbial growth during storage, thereby confirming the adequate dryness of the obtained material. (Ojike et al. 2020). Maceration of the simplicial in 96% ethanol produced 132.7 g of crude extract, corresponding to an extraction yield of 18.96%. Phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, and terpenoids. Fractionation yielded 42.7 g of ethyl acetate fraction. TLC analysis was conducted to characterize the metabolites in the fraction. The ethyl acetate fraction, analyzed using n-hexane: chloroform (1.5:3.5), showed an Rf of 0.6 with greenish-yellow spots, consistent with the presence of flavonoids as the major semi-polar constituents.

The phytochemical characterization of the ethyl acetate fraction has been reported in our previous study (winartiana et al. 2024) The ^{1}H-NMR analysis revealed the presence of aromatic signals at δ 5.6–7.3 µg/mL and olefinic signals at δ 5.1–5.4 µg/mL, indicating the presence of flavonoid and terpenoid compounds. These metabolites are widely reported to possess antioxidant activity and protective effects on pancreatic cells, thereby supporting the use of this fraction in the present study to evaluate cytotoxic and antidiabetic activities (Lee et al. 2020).

Cytotoxicity Profile on HepG2 Cells

The cytotoxicity evaluation using HepG2 cells was performed as a preliminary assessment of general cellular toxicity and does not represent pancreatic-specific biological effects. The ethyl acetate fraction

of the pineapple crown contains flavonoid secondary metabolites with potential antidiabetic activity. However, information regarding its toxicity remains limited. Therefore, an in vitro cytotoxicity assay was performed using the MTT method on HepG2 cells, with Doxorubicin HCl used as a positive control.

Both Doxorubicin HCl and the ethyl acetate fraction exhibited concentration-dependent inhibition of cell viability. Doxorubicin HCl showed strong cytotoxic activity with an IC_{50} value of 43.62 µg/mL, whereas the ethyl acetate fraction demonstrated markedly lower cytotoxic potency, with an IC_{50} value of 287.14 µg/mL. A statistically significant inhibitory effect of the fraction was first observed at 175 µg/mL (25% inhibition) and increased to 70% inhibition at 500 µg/mL. At the highest tested concentration (1000 µg/mL), the fraction inhibited cell viability by 88% (Figure 1).

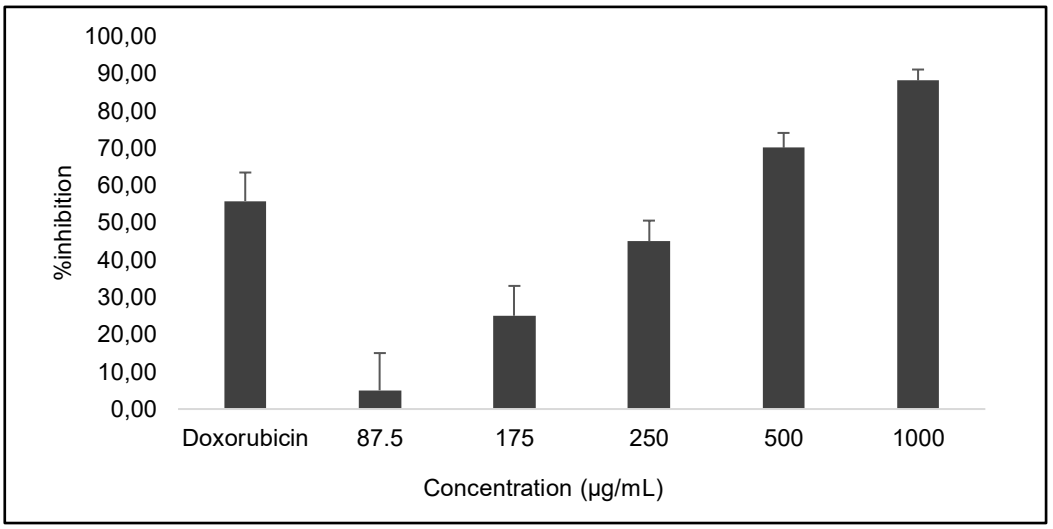

Figure 1. Percentage inhibition of cell viability induced by Doxorubicin HCl and the ethyl acetat fraction at various concentrations. Data are presented as mean ± SD. ($p < 0.001$) among treatment consentration.

Statistical analysis confirmed that the data were normally distributed and homogeneous ($p > 0.05$). One-way ANOVA revealed a significant difference in cell viability inhibition among treatment concentrations ($p < 0.001$), and Tukey's post hoc test indicated that higher concentrations (>175 µg/mL) significantly increased inhibitory activity compared to lower concentrations.

Comparative analysis indicated that the IC_{50} value of the ethyl acetate fraction was approximately 6.58-fold higher than that of doxorubicin, confirming its substantially lower cytotoxic potency relative to the positive control. However, caution is required in interpreting this as low toxicity, as doxorubicin is a highly potent chemotherapeutic agent. Furthermore, the substantial inhibition observed at higher concentrations (up to 88% at 1000 µg/mL) indicates that the fraction still exhibits significant cytotoxic effects; therefore, its safety profile should be interpreted with caution.

When compared with literature data, the IC50 value of the ethyl acetate fraction was higher than that of isolated pure flavonoids, such as 5.7-dimethoxyflavone, herbacetin, and apicathecin, but lower than that of the crude pineapple crown extract reported previously (Valderrama et al. 2022; Aeni et al. 2022; Rebai et al. 2025). Accordingly, the ethyl acetate fraction of pineapple crown can be classified as exhibiting moderate cytotoxic activity, more active than crude extracts but less potent than isolated pure flavonoid compounds.

Morphological Changes of HepG2 Cells

The effects of the test sample on HepG2 cell morphology were evaluated using phase contrast microscopy, as shown in Figure 2. The Doxorubicin HCl-treated

group used a positive control (Figure 2A) exhibited marked cytotoxic features, including cell shrinkage, loss of adherence, and reduced cell density. Treatment with the test sample at 87.5 µg/mL (Figure 2B) resulted in minimal morphological alterations, with most cells maintaining normal shape and attachment. At 175 µg/mL (Figure 2C), early signs of cytotoxicity became evident, characterized by partial cell rounding and decreased cell-cell contacts. More pronounced morphological damage was observed at 250 µg/mL (Figure 2D), followed by extensive cell detachment and fragmentation at 500 µg/mL (Figure 2E). At the highest concentration tested, 1000 µg/mL (Figure 2F), HepG2 cells exhibited severe morphological alterations comparable to those observed in the Doxorubicin HCl-treated group.

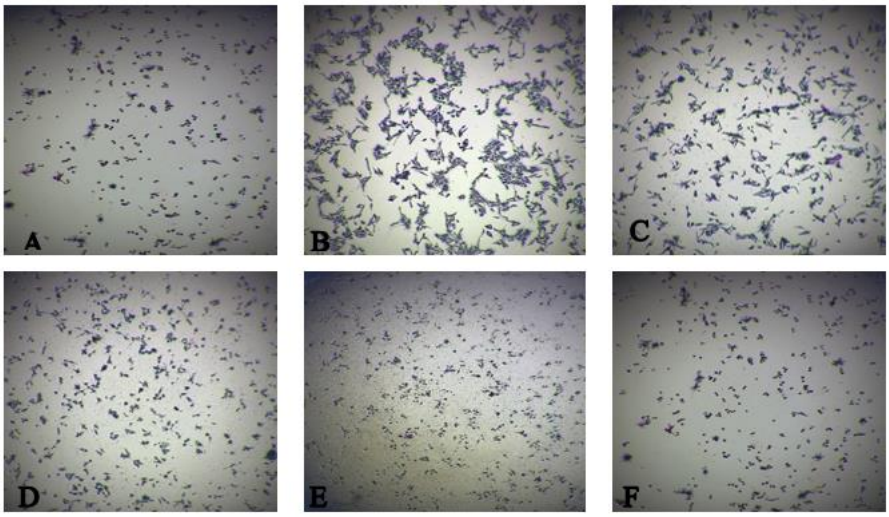

Figure 2. Morphological changes of HepG2 cells after treatment
Photomicrographs were captured at 400x magnification and representative of the experiment conditions. (A) Doxorubicin HCl-treated HepG2 cells, (B) HepG2 cells treated with 87.5 µg/mL; (C) 175 µg/mL; (D) 250 µg/mL; (E) 500 pµg/mL; and (F) 1000 µg/mL of the test sample.

Overall, Figure 2 demonstrates a concentration-dependent cytotoxic effect of the test sample on HepG2 cells. The observed morphological alterations, such as cell shrinkage, rounding and detachment, are well-recognized indicators of cellular damage induced by cytotoxic compounds and are commonly used as supportive parameters in in vitro cytotoxic evaluation (Malhão et al. 2022; Thusyanthan et al. 2022; Xu et al. 2023). These morphological findings are also consistent with the results of the call viability assay, thereby strengthening the reliability of the observed biological response and confirming that the detected cytotoxic effects reflect genuine cellular response(Rekha and Anila 2019).

In vivo Antidiabetic Activity (Physiological and Pancreatic Effects)

The following results represent systemic antidiabetic effects observed in vivo and cannot be directly inferred from in vitro cytotoxicity findings.

Blood glucose levels

Body weight increased significantly following HFD feeding, indicating the development of obesity and insulin resistance, and decreased markedly after alloxan induction, reflecting a catabolic metabolic state. Body weight increased significantly after HFD feeding ($p < 0.05$), Table 1. Administration of the tested fraction resulted in a gradual recovery of body weight, suggesting an improvement in metabolic status. This effect may be associated with enhanced insulin action and glucose utilization, contributing to better energy balance and tissue anabolism. These observations are consistent with previous studies reporting similar effects of flavonoid-rich plant extract, such as *Physalis minima* L., on metabolic regulation in diabetic models (Valderrama et al. 2022).

Table 1. Average Body Weight of Rats at Different Experimental Stages

No	Experimental groups	Normal	After Aloksan	After Fraction
1	Negative control	238 ± 29	274 ± 24	260 ± 29
2	Positive control	246 ± 45	296 ± 45	298 ± 43
3	Dose 10mg/kgBW	232 ± 36	278 ± 47	252 ± 37
4	Dose 15 mg/kgBW	248 ± 23	294 ± 19	282 ± 18
5	Dose 18 mg/kgBW	230 ± 20	270 ± 22	243 ± 26
6	Dose 22 mg/kgBW	220 ± 16	278 ± 13	283 ± 22
7	Dose 25 mg/kgBW	245 ± 19	300 ± 34	263 ± 59

Data are presented as mean ± SD (n = 5).

All experimental rats exhibited normal baseline blood glucose levels (86-132 mg/dL). Induction with alloxan successfully produced hyperglycemia, as evidenced by elevated blood glucose levels in the positive control and all treatment groups, confirming the establishment of a diabetic model. Following administration of the tested fraction, a reduction in blood glucose levels was observed across the treatment groups, although the response did not follow a linear dose-response pattern Picture 3. Among the tested doses, the 22 mg/kgBW group demonstrated the optimal antihyperglycemic effect, reducing blood glucose levels to near-normal values (102 ± 14 mg/dL). Statistical analysis confirmed that this reduction was significant compared with the diabetic control group ($p<0.05$).

Although the 25 mg/kgBW dose produced the largest absolute decrease in blood glucose levels, the final glucose concentration remained above the normal physiological range. This finding suggests the occurrence of a plateau or non-linear dose response effect, in which increasing the dose does not necessarily result in improved glycemic control (Ezeigbo and Asuzu 2011; Pottathil et al. 2020). Similar phenomena have been reported in previous studies involving plant-derived extracts of *Ficus asperifolia* leaves, where higher doses failed to confer additional therapeutic benefits and, in some cases, reduced efficacy (Pwaniyibo et al. 2020)

In contrast, the lowest tested dose exhibited minimal antihyperglycemic activity, indicating that subtherapeutic dosing is insufficient to counteract alloxan-induced metabolic dysfunction. Taken together, these findings demonstrate that the antihyperglycemic activity of the tested fraction does not follow a linear dose-response relationship, with the optimal therapeutic effect observed at 22 mg/kgBW.

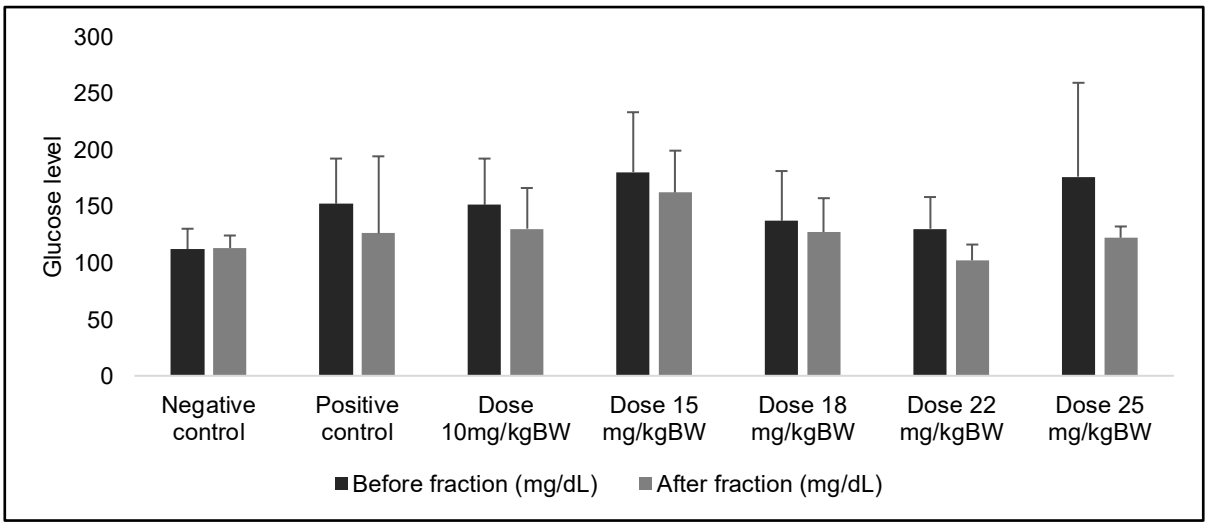

Figure 3. Blood glucose levels of rats post alloxan induction and post treatment. Data are presented as mean ± SD.

Pancreatic Islet Cell Analysis (α + β)

Induction of diabetes using an HFD combined with alloxan resulted in a significant increase in pancreatic α-cell dysregulation associated with diabetic conditions and reflects a compositional shift within the islets of Langerhans following β-cell impairment, as previously reported (Ifedigbo et al. 2016; Wickramasinghe et al. 2024). The mean pancreatic α-cell counts in each experimental group are presented in Table 2. Administration of pineapple crown ethyl acetate fraction at doses of 10, 15, and 18 mg/kgBW did not effectively reduce α-cell numbers, as indicated by relatively high mean values (50±1.5, 48±1.5, and 47± respectively). The positive control group showed a mean.

Table 2. Mean Pancreatic α-Cells Counts in Diabetic Rats Model Under Various Antidiabetic Treatments

No	Experimental groups	α-Cells
1	Negative control	34 ± 2.3
2	Positive control	41 ± 1.4
3	Dose 10mg/kgBW	50 ± 1.5
4	Dose 15 mg/kgBW	48 ± 1.5
5	Dose 18 mg/kgBW	45 ± 1.7
6	Dose 22 mg/kgBW	40 ± 1.7
7	Dose 25mg/kgBW	43 ± 1.4

Data are presented as mean ± SD (n = 5). Kruskal–Wallis analysis showed a significant difference among groups ($p < 0.05$), but no significant pairwise differences were observed in the Mann–Whitney U test.

The positive control group treated with a standard antidiabetic drug showed an α-cell count of 41±1.4. The lowest α-cell count was observed in the 22 mg/kgBW group (40±1.7), followed by the 25 mg/kgBW group (43±1.4). Statistical analysis using the Kruskal–Wallis test showed a significant difference among groups ($H = 28.608$, $df = 6$, $p < 0.001$). However, post hoc analysis using the Mann–Whitney U test revealed no statistically significant differences between individual groups. This indicates that although overall group differences were present, pairwise comparisons did not show sufficiently strong effects, possibly due to within-group variability or the relatively small sample size.

The reduction in α-cell count at the 22 mg/kg BW dose suggests that this concentration represents an optimal therapeutic level capable of modulating α-cell activity. This finding is consistent with previous studies demonstrating improved pancreatic function and partial recovery of β -cell activity at comparable doses (Dusaulcy et al. 2016; Almuttairi 2023). However, increasing the dose to 25 mg/kgBW appeared to attenuate this effect, suggesting a possible supra-optimal response at higher concentrations that may reduce therapeutic efficacy.

In addition to α-cell changes, the effect of treatment on pancreatic β-cells was also evaluated. As shown in Table 3, the mean β-cell counts across groups were 61 ± 2.3 (negative control), 40 ± 1.5 (positive control), 22 ± 1.5, 28 ± 1.5, 40 ± 1.7, 48 ± 1.7, and 43 ± 1.7 for the 10, 15, 18, 22, and 25 mg/kgBW dose groups, respectively. The data were normally distributed and homogeneous ($p > 0.05$). One-way ANOVA revealed a significant difference in β-cell counts among groups ($F = 265.981$, $p < 0.001$). Post hoc analysis using Tukey's test revealed significant differences among the treatment groups.

Table 3. Mean Pancreatic β-Cells Counts in Diabetic Rats Model Under Various Antidiabetic Treatments

No	Experimental groups	β cells
1	Group 1 (Negative control)	61 ± 2.3
2	Group 2 (Positive control)	40 ± 1.5

No	Experimental groups	β cells
3	Group 3 (Dose 10mg/kgBW)	22 ± 1.5
4	Group 4 (Dose 15 mg/kgBW)	28 ± 1.5
5	Group 5 (Dose 18 mg/kgBW)	40 ± 1.7
6	Group 6 (Dose 22 mg/kgBW)	48 ± 1.7
7	Group 7 (Dose 25 mg/kgBW)	43 ± 1.7

Data are presented as mean ± SD (n = 5). One-way ANOVA showed significant differences among groups ($p < 0.05$).

A dose-dependent increasing trend in β-cell count was observed from 10 to 22 mg/kgBW, with the highest value at 22 mg/kgBW, followed by a decrease at 25 mg/kgBW. This pattern indicates a non-linear dose-response relationship, possibly due to receptor saturation or inhibitory effects at higher concentrations, where excessive stimulation may reduce cellular responsiveness and limit β-cell regeneration (Huber 2013)From a biological perspective, β-cells play a crucial role in insulin secretion and glucose regulation; therefore, the observed increase in β-cell number may indicate partial improvement in pancreatic endocrine function. However, the β-cell count at the optimal dose remained lower than that of the normal control group, indicating that full recovery was not achieved.

These findings complement the observed modulation of α-cell counts, suggesting a coordinated effect on pancreatic islet cell composition. The reduction in α-cell count, together with the increase in β-cell number at the optimal dose, suggests a potential shift toward improved islet homeostasis under diabetic conditions. However, this interpretation should be made cautiously, as insulin levels and functional β-cell activity were not directly measured in this study.

Furthermore, the consistent pattern observed in both α- and β-cell responses supports a non-linear dose–response relationship, with an optimal effect at 22 mg/kgBW and reduced efficacy at higher doses.

Integrated discussion

The present study demonstrates that the ethyl acetate fraction of pineapple crown exhibits biological activity in two distinct experimental systems. The in vitro cytotoxicity assay using HepG2 cells provides a general assessment of hepatic cellular toxicity, whereas the in vivo experiments reflect systemic metabolic regulation and pancreatic endocrine responses in diabetic conditions.

These two experimental models assess different biological endpoints. Therefore, the cytotoxicity findings in HepG2 cells cannot be directly extrapolated to pancreatic outcomes, and no causal or mechanistic relationship can be inferred between the two datasets. Each model provides independent, yet complementary, information on the safety profile and antidiabetic potential of the tested fraction.

This study has several limitations. First, the absence of mechanistic investigations limits the understanding of the molecular pathways underlying the observed antidiabetic effects. Second, the lack of insulin analysis-related measurements restricts the comprehensive interpretation of pancreatic endocrine function. Additionally, using HepG2 cells provides only a general cytotoxicity assessment and does not directly reflect pancreatic cellular responses.

Future studies are recommended to include insulin quantification, β-cell functional assays, and the use of pancreatic cell models such as INS-1 or MIN6 cells. Further molecular investigations focusing on oxidative stress and glucose metabolism pathways are also required to clarify the mechanism of action of the ethyl acetate fraction.

CONCLUSION

The ethyl acetate fraction of pineapple crown exhibited lower cytotoxicity against HepG2 cells, with an IC_{50} of 287.14 µg/mL, approximately 6.58 times higher than that of the positive control, Doxorubicin HCl (IC_{50} = 43.25 µg/mL). This indicates a relatively weaker cytotoxic potential compared to the reference chemotherapeutic agent. In the in vivo model, a high-fat diet combined with

alloxan successfully induced diabetic conditions, as indicated by weight loss and hyperglycemia. Treatment with the ethyl acetate fraction improved physiological parameters, particularly at a dose of 22 mg/kgBW, which significantly reduced blood glucose levels compared to the diabetic control group. In addition, this dose was associated with a reduction in pancreatic α-cell counts toward near-normal levels and an increase in β-cell numbers, suggesting a potential improvement in pancreatic islet cell composition under diabetic conditions. Overall, these findings indicate that the ethyl acetate fraction of pineapple crown possesses potential antidiabetic activity in vivo, while demonstrating moderate cytotoxicity in vitro. However, these effects were observed in different experimental systems and should be interpreted as distinct biological outcomes rather than directly mechanistically linked phenomena.

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