

Research article

Pancreatic protective effect of ethanolic extract of Mengkudu (*Morinda citrifolia*) on rats induced by doxorubicin

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Abstract

Cancer is a disease or abnormality in the body due to body cells that grow and develop abnormally and beyond normal and very wild limits and doxorubicin is used in cancer therapy, but produces reactive oxygen species (ROS) which are toxic to pancreatic beta cells and made pancreatic not properly produced insulin can causes increasing of glucose level. The purpose of this study was to determine the protective pancreatic activity of mengkudu fruit ethanol extract against rats induced with doxorubicin. Mengkudu fruit ethanol extract was obtained by maceration. The protective pancreatic activity test is carried out by measuring blood glucose and blood HbA1c levels. Animals were induced with doxorubicin (DOX) 5 mg/kgbw on day 1, 7, 14 and 20th. and blood glucose is checked on the first day before doxorubicin-induced and afterward on days 5, 10, 15, and 20. Administration of mengkudu extract 100 mg/kg BW, 300 mg/kgbw, and 500 mg/kgbw given starting from day 1 to day 20 and on the 21st-day blood was taken then HbA1c levels were examined. The results obtained of ethanol extract of mengkudu fruit dose of 100 mg/kgbw, 300 mg/kgbw, and 500 mg/kgbw had a significant decrease in blood glucose levels ($P < 0.05$) with a negative control group that was only given CMC-Na and doxorubicin. Ethanol extract of mengkudu fruit dose of 100 mg/kg BW, 300 mg/kgbw, and 500 mg/kgbw had a significantly reduced activity of HbA1c levels ($P < 0.05$) with a negative control group that was only given CMC-Na and doxorubicin. Enhancement mengkudu does increase in rats given doxorubicin resulted in improved pancreatic beta cells and decreased blood glucose and HbA1c.

Introduction

Cancer is a disease or abnormality in the body resulting from body cells that grow and develop abnormally and beyond normal and very wild limits. Cancer is also one of the leading causes of death throughout the world. Globocan data states that in 2018 there were 18.1 million new cases with a mortality rate of 9.6 million deaths, where 1 in 5 men and 1 in 6 women in the world experience cancer. The data also states that 1 in 8 men and 1 in 11 women die from cancer. The incidence of cancer in Indonesia has a prevalence of cancer of 136.2 / 100,000 population and ranks 8th in Southeast Asia, whereas in Asia it is 23rd. The highest incidence rate in Indonesia for men is lung cancer which is 19.4 per 100,000 the population with an average death of 10.9 per 100,000 population, followed by liver cancer of 12.4 per 100,000 population with an average death of 7.6 per 100,000 population [1].

Anthracycline doxorubicin is a very effective anti-neoplastic agent, which intercalates in DNA and inhibits topoisomerase II. Doxorubicin is one of the most common systemic treatments for increasing several cancers in adults and also children, including

hematologists and solid tumors. Unfortunately, Doxorubicin's clinical efficacy is hampered by dose-related toxicity, such as hematopoietic suppression and organ toxicity; although the most serious side effect is life-threatening cardiomyopathy, other organs are also involved, such as the liver, kidneys, and pancreatic [2-3]. The pancreas has an important function for the body; regulates the metabolism of carbohydrates, proteins, and fats, with these four polypeptides which are regulatory activities released by the Langerhan islands in the pancreas. Two of them, insulin and glucagon which are important hormones in terms of metabolism. The third polypeptide, somatostatin, plays a role in the regulation of the secretion of langerhan island cells. The fourth polypeptide is related to the regulation of ions in the intestine. Insulin is anabolic, increasing the storage of glucose, fatty acids, and amino acids. Glucagon is catabolic, mobilizing glucose, fatty acids, and amino acids from storage into the bloodstream. Thus, these two hormones are reciprocal in their overall actions and are reciprocally secreted in most circumstances. Excess insulin causes hypoglycemia, which causes seizures and coma. Insulin deficiency, both absolute and relative, causes diabetes mellitus (chronic increase in blood

glucose), a complex and debilitating disease which, if left untreated, is ultimately fatal. Glucagon deficiency can cause hypoglycemia, and excess glucagon makes diabetes worse. Overproduction of pancreatic somatostatin causes hyperglycemia and other manifestations of diabetes [4].

Exposure to chemotherapeutic agents has been associated with an increased risk of type 2 diabetes (T2D = Type 2 diabetes), a disease characterized by peripheral insulin resistance and impaired glucose secretion stimulated insulin (GSIS = Glucose-stimulated insulin secretion) from pancreatic β cells. In a study conducted by heart using INS-1 β mouse line 832/13 and isolated pancreatic islet mice, and investigated the effect of doxorubicin (Adriamycin) chemotherapy drugs on pancreatic β cell survival and function. Exposure of INS-1 cells 832/13 to doxorubicin causes a decrease in GSIS (Glucose stimulated insulin secretion), cellular viability, increased cellular toxicity, immediately after 6 hours after exposure. Doxorubicin interferes with plasma membrane electron transport (PMET), the pathway depends on the reduction of NADH (Nicotinamide adenine dinucleotide + Hydrogen) and NADPH (Nicotinamide adenine dinucleotide phosphate) but fails to reduce the redox cycle in INS-1 cells 832/13. Although NADPH / NADP (+) content is not affected, NADH / NAD (+) content decreases at 4 hours post-exposure to doxorubicin and is followed by a reduction in ATP (Adenosine triphosphate) content. Previous studies have shown that doxorubicin functions as a topoisomerase II inhibitor through the induction of DNA crosslinking, producing apoptosis. Doxorubicin induces mRNA expression for mdm2, cyclin G1, and fas while decreasing the regulation of p53, and increasing the melting temperature of genomic DNA, consistent with DNA damage and induction of apoptosis. Doxorubicin also induces caspase-3 and -7 activity in INS-1 cells 832/13 and rat islands; concomitant treatment with Z-VAD-FMK (cell-permeable fluoromethyl ketone-derivatized peptides) pan-caspase inhibitors temporarily weakens the loss of viability mediated by doxorubicin in INS-1 cells 832/13. Together, these data show that DNA damage, not H₂O₂ produced through the redox cycle, is the main mechanism of doxorubicin toxicity in pancreatic β cells [5].

Mengkudu (*Morinda citrifolia* L = Mengkudu) has been used extensively as a complementary and alternative therapy in many countries because of its strong antioxidant activity and proven health benefits. Mengkudu is traditionally used as a therapeutic drug for various diseases such as antibacterial, antitumor, anthelmintic, analgesic, anti-inflammatory, immunostimulant, gastritis, skin diseases, respiratory infections, menstrual disorders, and urinary tract disorders. Mengkudu fruit contains hydrophilic compounds such as carbohydrates, proteins, minerals, vitamins and less fat [6], and is rich in alkaloids called xeronin. These alkaloids are useful for activating

enzymes and regulating the formation of proteins and work to fight inflammation that occurs in the body. Xeronin is formed by a substance called proxeronin and is produced when the stomach acid digesting the mengkudu fruit converts proxeronin to xeronin. All cells entered by xeronin will become active, healthier, and the structure and function will improve. The need for xeronin tends to increase if there are health problems (both physical and emotional), infections, toxins, and increasing age. Mengkudu fruit also contains scopoletin which functions to widen blood vessel channels and facilitate blood circulation and has efficacy as an anti-bacterial, anti-allergic and anti-inflammatory [7-9].

Based on the background of providing cancer therapy with doxorubicin, it often causes organ toxicity, and Mengkudu fruit containing rich phenols and flavonoids that are strong anti-free radicals, thus encouraging researchers to test the pancreatic protective activity of Mengkudu fruit ethanol extract against doxorubicin-induced experimental animals by measuring the biochemical parameters of blood glucose level and HbA1c.

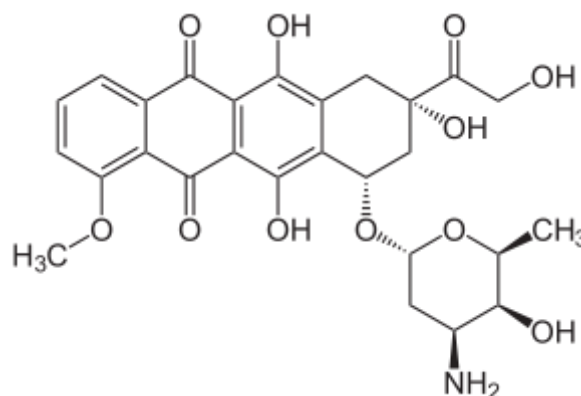


Figure 1. Structure of doxorubicin.

Materials and methods

Material

Microplate Reader, pH meter (OHAUS Starter300 Portable) Beaker glass (IWAKI CTE33), Multiskan Go Reader (Thermo Fisher Scientific 1510), analytic measure, Eppendorf tube, Vial 1 ml, Spatula, Micropipet (1-10 μ L, 50-200 μ L, 100-1000 μ L) (Eppendorf), Thermometer, automated plate washer, Extract ethanol *morinda citrifolia*, Ketamine (Sigma P-4417), Doxorubicin (Merck 109057), CMC-Na (Sigma P-4417).

Animals

Animals used in research are a rat (*Rattus norvegicus*) wistar male 150 – 200 g. Before the study began, test animals were adjusted for one week with the condition of the room temperature (22-25°C), under the cycle of 12 hours light/ dark, given the food and the drinking water.

Ethics Commission from health and science commission, University of Sumatera Utara. Animal Ethical Number is 0523/KEPH-FMIPA/2019.

Preparation of ethanol extract of Mengkudu

Air-dried leaves of mengkudu (*Morinda citrifolia*) (800g) were extracted with 90% ethanol (12L) three times (2h each) using a soxhlet under reflux. The ethanol extract was concentrated under vacuum to give a crude extract (150g).

Phytochemical screening of ethanol extract of Mengkudu

Phytochemical screening of extract ethanol Mengkudu method consisted of identification of phenol, steroids/terpenoids, saponins, flavonoids, tannin and alkaloids.

In vivo test cardioprotective effect of ethanolic extract of mengkudu

In vivo tested in an experiment by using 24 Wistar rats (*Rattus norvegicus*) male and weight 150 g - 200 g, as many as 24 and divided into 6 groups and each group consisted of 4 rats :

Normal : Suspension Sodium- Carboxymethylcellulose (Na-CMC).

Negative control : Wistar rats (*Rattus norvegicus*) male induced by doxorubicin 15 mg/kgbw.

Positive control : Wistar rats (*Rattus norvegicus*) male induced by doxorubicin 15 mg/kgbw + Vitamin E 1%.

Group 1 : Wistar rats (*Rattus norvegicus*) male induced by doxorubicin 15 mg/kgbw + 100 mg/kgbw.

Group II : Wistar rats (*Rattus norvegicus*) male induced by doxorubicin 15 mg/kgbw + 300 mg/kgbw.

Group III : Wistar rats (*Rattus norvegicus*) male induced by doxorubicin 15 mg/kgbw + 500 mg/kgbw.

Rat induced by doxorubicin with an accumulative dose of 20 mg/kgbw over for 21 days, with the dose of administered 5 mg/kgbw in day 1, 7, 14, 20. Before treatment, the rats adapted for 14 days and then continued with the administered of doxorubicin and the treatment of rats for 21 days administered by the extract with a dose of 100 mg/kgbw, 300 mg/kgbw, 500 mg/kgbw. Blood glucose level was examined in day 1, 5, 10, 15, 20. On the last day of treatment the rats fasting 18 hours before performed surgery on the animal test. Rats administered by ketamine 70 mg/kgbw an intraperitoneal then continued to the surgery. Thoracic dissected and the blood was taken as much as 3 ml. The blood transferred into a microtube. Then the blood is examined for HbA1c level [10].

Determination of HbA1c

HbA1c can be measured by the immunoassay method. Specimens used for measurement of HbA1c are capillary

or venous blood with anticoagulants (EDTA, citrate, or heparin). Avoid hemolysis during sample collection [11].

Statistical analysis

Test analysis was carried out by using one-way analysis of variance (ANOVA) followed by Post Hoc Test using the Tukey HSD test. $P < 0.05$ was considered as statistical significance and also use IBM SPSS 20.

Result and discussion

Authentication of plant

The results of the identification of plants carried out by Elisabeth (2019) at the Medanese Herbarium (MEDA) the University of North Sumatra, the fruit used in this study was Mengkudu (*Morinda citrifolia*). Kingdom : Plantae, Subkingdom: Tracheobionta, Super Division : Spermatophyta, Division : Magnoliophyta, Class : Magnoliopsida, Subclass : Asteridae, Ordo : Rubiales, Familiy : Rubiaceae, Genus : Morinda, Species : *Morinda citrifolia* L.

Phytochemical tests

The results of phytochemical screening qualitatively in Mengkudu extract are shown in table 1.

Table 1. Results of phytochemical screening of mengkudu extract.

Chemical Component	Result
Tanin	+
Saponin	+
Flavonoid	+
Phenol	+
Steroid	+
Glikosida	+
Alkoholoid	+

Phytochemical screening of ethanol extract of mengkudu showed the positive result of flavonoids, tannins, saponins, glycosides, alkaloid, and steroids.

Another phytochemical screening for the presence of secondary metabolites is carried out on *morinda citrifolia* fruit extracts from India, ethanol and methanol extracts detect steroids, glycosides, phenols, tannins, terpenoids, alkaloids, carbohydrates, flavonoids, small sugars, lipids and fats in all types of extracts, whereas saponins in water and methanol extracts, and acidic compounds in water extracts only [11]. Brazil *morinda citrifolia* fresh fruit pulp also shows the presence of small amounts of glucose, fructose and sucrose, and contains a large amount of minerals [12]. Recently, phytochemical screening of different commercial Nigerian *Morinda citrifolia* juice extracts confirmed the presence of secondary metabolites such as reducing sugar, phenol, tannin, flavonoids, saponins, glycosides, steroids, terpenoids, alkaloids, and acidic components, and this

study also reported no the presence of anthraquinone, pilhanin, and resin [12]. Brazilian aqueous extracts from a phytochemical screening of *Morinda citrifolia* leaves show the presence of alkaloids, coumarin, flavonoids, tannins, saponins, steroids, and triterpenoids [13]. Phytochemical studies of dichloromethane extracts from *Morinda citrifolia* from Malaysia have resulted in the isolation and characterization of ten anthraquinones including I-hydroxy-2-methylanthraquinone, nordamnacanthal, damnacanthal, morindone, rubiadin-I-methyl ether. Furthermore filtering of dried *Morinda citrifolia* seeds from China resulted in the identification of twenty compounds from ethanol extracts, including daucosterol, ursolic acid, 19-hydroxyursolic acid, 1,5,15-trimethylmorindol, 5,15-dimethylmorindol, scopoletin, 3,30-bisdemethylpinoresinol, 3,4,30,40-tetrahydroxy-9,70a-epoxylignano-7a, 90-lactone, americanin D, americanin A, americanin, isoprincepin, deacetyl-asperulosidic acid, loganic acid, asperulosidic acid, rhodolatoside, quercetin-3-O- α -L rhamnopyranosyl- (1 β 6)- β -D-glucopyranide- hydroxyl-succinate, 5-hydroxy methyl-2-furancarboxaldehyde, 3-methylbut-3-enyl-6-ObD-glucopyranosyl- β -D-glucopyranoside [14]. For a novel and accurate result, the quality of *M. citrifolia* studies can be improved through the development of identification markers for their chemical components and

characterizing their chemical structures in TLC, HPTLC, HPLC, NMR spectroscopy [15].

Blood glucose level

In this research, conducted an examination of blood glucose level from the blood of rats. Results obtained can be seen in table 2.

The data in table 2 showed that after rat have fasted for 18 hours then fasting blood sugar levels were measured and immediately given doxorubicin at a dose of 5 mg/kgbw. Then blood sugar checks were calculated on the first day, from the table shows that all groups induced by doxorubicin at a dose of 5 mg/kgbw had the highest increase in blood sugar and blood sugar was the negative group that was not given protection so that there was a significant increase ($P < 0.05$) when compared to the normal, positive, treatment group I, treatment group II and treatment group III. The positive control treatment group did not differ significantly ($P > 0.05$) from the normal control group, the treatment group I, treatment group II, and treatment group III while the negative control group was significantly different ($P < 0.05$). Administration of vitamin e and extracts provide a protective effect against beta pancreas cells so that they can produce insulin. The data can be seen in the figure 2.

Table 2. Blood glucose level on first day.

No.	Doses	Mean Blood Glucose Level on First Day \pm SD (mg/ml)	
		Before induced Doxorubicin	After induced Doxorubicin
1.	Normal	75.4 \pm 12.3	75.4 \pm 11.6
2.	Negative control	74.2 \pm 10.4	243.4 \pm 11.3
3.	Positive control	72.4 \pm 11.2	156.7 \pm 14.9
4.	Group I	72.1 \pm 12.1	200.9 \pm 12.5
5.	Group II	76.3 \pm 13.2	199.5 \pm 13.8
6.	Group III	73.6 \pm 10.2	183.2 \pm 14.4

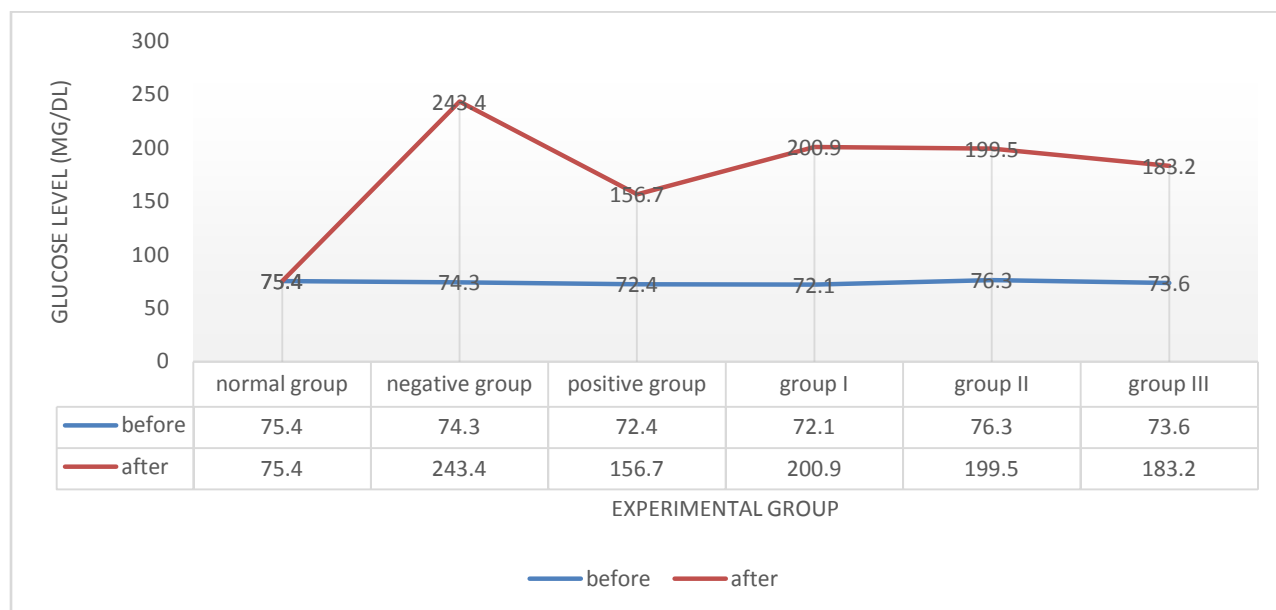


Figure 2. Blood glucose Level on first day before and after induced doxorubicin.

Blood glucose level on day 1, 5, 10, 15, and 20

Each group included normal group, positive control group, negative control group, treatment group I, treatment group II, and treatment group III were treated with doxorubicin with a dose of 5 mg/kgbw which was then given on days 1, 7, 14, and 21, and carried out checking the blood glucose level on days 1, 5, 10, 15, and 20. The data can be seen in the table 3.

The data presented in the form of Mean ± SD. Data obtained results based on the results of the statistical tests, the levels of blood glucose on negative control normal have a significant difference (p < 0.05) with normal, positive control, treatment group I, II, III.

Based on the table 3 it can be seen a decrease in blood sugar levels in the positive control group, treatment group I, treatment group II, and treatment group III, while the negative control group that was only given CMC without protection experienced a significant increase in blood sugar levels reached 568.5 mg/dl this indicates that treatment animals that are only given doxorubicin have damaged pancreatic cells, so that the pancreatic beta cells cannot produce enough insulin. In addition, you can see the figure 3.

Measurement of HbA1c

The measurement of HbA1c was carried out using the Rat HbA1c Kit by the ELISA method, which was read for absorbance with a microplate reader at a wavelength of 450nm. This method is based on the principle of measuring antigens or antibodies that are both relative

and quantitative. The level of HbA1c can be seen in table 4.

The data above shows that there was a decrease in HbA1c purification levels in each treatment group, the normal group that was only given CMC average HbA1c concentration was 24.8 ± 0.412 (ng/ml), while the negative group given CMC and doxorubicin had high HbA1c levels ie 69.67 ± 0.894 (ng/ml), the positive control group given Doxorubicin with Vitamin e showed a decrease in HbA1c levels of 26.7 ± 0.267 (ng/ml), whereas in the treatment group I obtained results with a dose of 100 mg/kg BW which is 39.8 ± 0.673 (ng/ml) while in the treatment group II with a dose of 300 mg/kg BW the results obtained were 36.2 ± 0.375 (ng/ml), and the treatment group III with a dose of 500 mg/kg BW obtained the results were 28.9 ± 0.210 (ng/ml).

Statistically, it was found that the negative control group had a significant difference (P <0.05) with all groups namely the normal group, the positive control group, the treatment group I, the treatment group II, and the treatment group III. The normal group had a significant difference (P <0.05) with the negative control group, the treatment group I, and the treatment group II, did not have a significant difference (P> 0.05) with the positive control group, the treatment group III. The positive control group had a significant difference (P <0.05) with the negative control group, the treatment group I, and the treatment group II, not having a significant difference (P> 0.05) with the normal group and treatment group III. The data above can be seen in figure 4.

Table 3. Blood glucose level.

No.	Doses	Blood Glucose Level ± SD (mg/ml)				
		Day 1	Day 5	Day 10	Day 15	Day 20
1.	Normal	75.4 ± 11.6	73.4 ± 10.6	78.3 ± 10.4	76.2 ± 9.8	78.3 ± 12.4
2.	Negative control	243,4 ± 11.3	273,4 ± 12.3	353.7 ± 9.4	483.4 ± 10.5	568.5 ± 13.4
3.	Positif control	156.7 ± 14.9	157.9 ± 9.10	121.2 ± 12.1	111.5 ± 12.8	98.4 ± 12.9
4.	Group I	200.9 ± 12.5	192.3 ± 10.5	187.3 ± 9.8	167.4 ± 10.1	150.3 ± 10.1
5.	Group II	199.5 ± 13.8	188.5 ± 14.1	168.3 ± 12.4	143.2 ± 12.3	128.3 ± 9.3
6.	Group III	183.2 ± 14.4	179.2 ± 12.1	156.3 ± 13.1	123.4 ± 9.1	101.8 ± 8.9

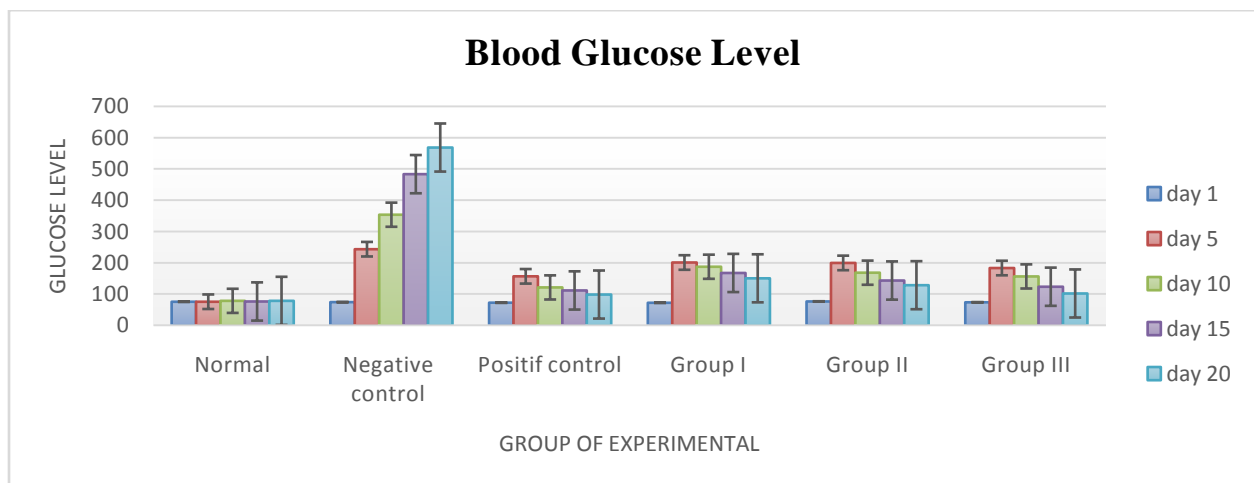
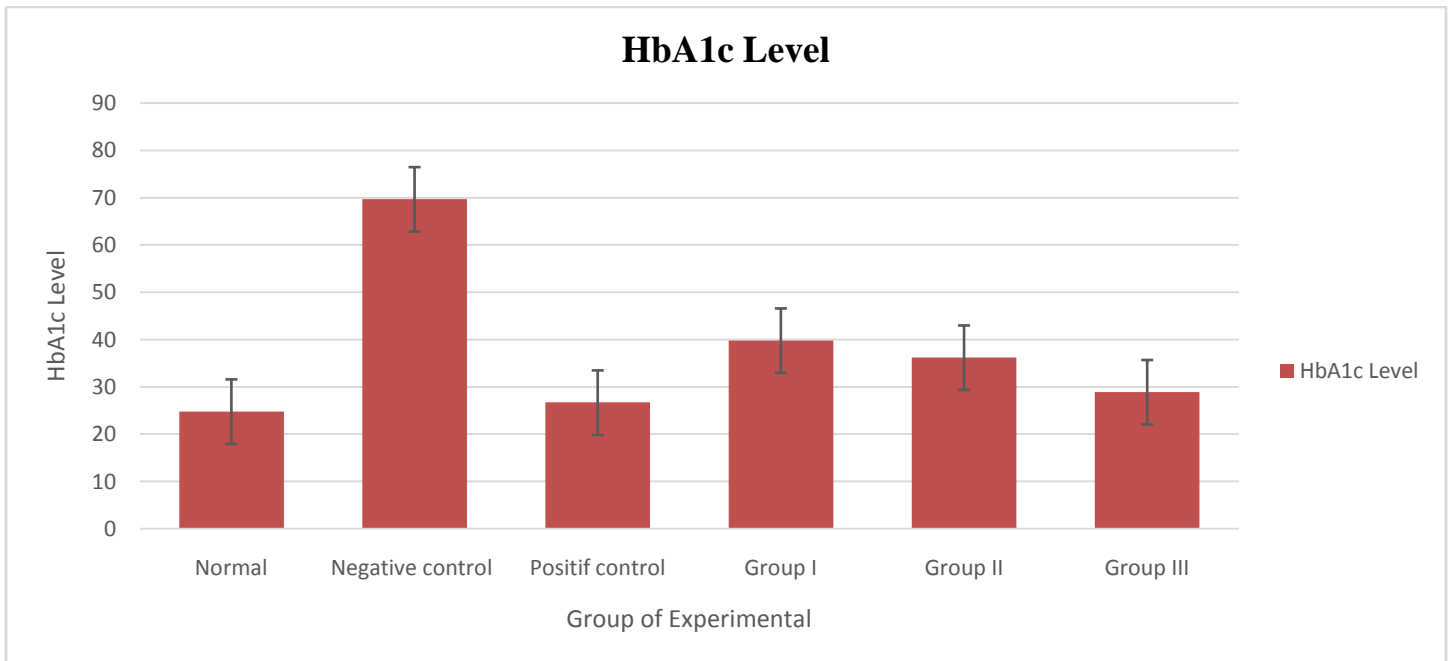


Figure 3. Blood glucose level on day 1, 5, 10, 15, and 20.

Table 4. HbA1c level.

No.	Doses	HbA1c Level \pm SD (ng/ml)
1.	Normal	24.8 \pm 0.412
2.	Negative control	69.67 \pm 0.894
3.	Positive control	26.7 \pm 0.267
4.	Group I	39.8 \pm 0.673
5.	Group II	36.2 \pm 0.375
6.	Group III	28.9 \pm 0.210

**Figure 4. HbA1c level.**

The results showed that the negative control group (DOX + CMC) was 69.67 ± 0.894 ng/ml showing an increase in HbA1c compared to the normal group. This is caused by the diabetogenic effect of doxorubicin which causes necrosis of β -pancreatic cells causing hyperglycemia [16]. When blood sugar levels are high, the non enzymatic glycation process will increase, where the glycation itself will cause the concentration of free radicals to also increase which triggers an increase in HbA1c levels [17].

Decreased HbA1c shows the effect of decreased blood glucose with increasing insulin secretion due to the regeneration of β -pancreatic cells. Flavonoids reduce blood glucose levels by inhibiting the enzyme α -glucosidase found in the small intestine. Inhibition of the enzyme α -glucosidase causes a decrease in the rate of digestion of carbohydrates into monosaccharides that can be absorbed by the small intestine, thereby reducing hyperglycemia. Decreased hyperglycemia contributes to decreasing HbA1c levels [18]. Another researcher have also suggested that after administration of the extract can

reduce fasting blood sugar levels, HbA1c in patients with type 2 DM.

The preventive effect of mengkudu extract may be caused by its ability to capture free radicals. Free radicals are atoms or molecules whose properties are very unstable and highly reactive, and damaged tissue. Antioxidants can reduce glucose level by preventing excessive oxidation so that pancreatic β cell damage can be prevented [20]. According to Younos *et al.* with the presence of antioxidants, the oxidation process due to free radicals can be slowed down, so that it will prevent degenerative diseases such as diabetes [21].

Treatment groups I, II, II contain bioactive compounds namely flavonoids, alkaloids, and tannins. Flavonoids and tannins are known to be able to play a role in capturing free radicals or function as natural antioxidants [22]. Antioxidants are involved in the process of repairing damaged cells. Cell damage caused by the presence of free radicals can be overcome by the presence of antioxidants that function to reduce oxidizers before damaging cells so that cell damage can be reduced. Antioxidants can

suppress β cell apoptosis without changing the proliferation of pancreatic β cells, to regenerate damaged β cells [23-25]. Alkaloid also can regenerate damaged pancreatic cells and increase insulin expenditure, so glucose level in the blood decreases [26].

Conclusion

Ethanol extract of mengkudu fruit dose of 100 mg/kgbw, 300 mg/kgbw, and 500 mg/kgbw have a significant decrease in blood glucose levels ($P < 0.05$) with a negative control group that is only given CMC-Na and doxorubicin. Mengkudu ethanol doses of 100 mg/kgbw, 300 mg/kgbw, and 500 mg/kgbw have significantly reduced activity of HbA1c levels ($P < 0.05$) with negative control groups that were only given CMC-Na and doxorubicin.

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