

PURIFIED GAMBIR (UNCARIA GAMBIR (HUNTER) ROXB.) ATTENUATE RENAL FUNCTION IN ALLOXAN-INDUCED DIABETIC MALE RATS

FITRI RACHMAINI¹, ARMENIA^{1*}, RAHMAD ABDILLAH¹, SITI NOOR ASYIKIN HAQQI²

¹Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Andalas, Limau Manis-25163, Padang, West Sumatera, Indonesia. ²Bachelor Program, Faculty of Pharmacy, Universitas Andalas, Limau Manis 25163, Padang, West Sumatera, Indonesia
*Corresponding author: Armenia; *Email: armenia@phar.unand.ac.id

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ABSTRACT

Objective: Diabetes and its vascular complications could prevent by antioxidants. Purified gambir is the purified extract of *Uncaria gambir* (Hunter) Roxb, which contains more than 90% catechins and scientifically work as antioxidant. This study aimed to determine the effect of purified gambir on renal function in alloxan-induced diabetic Wistar Kyoto rats.

Methods: Diabetic rats were induced by alloxan 125 mg/kgBW by intraperitoneal injection. Forty-five diabetic rats were divided into five groups, diabetic control group treated with vehicle, a positive control group treated with glibenclamide at dose 0.45 mg/kgBW, and treatment groups of purified gambir at doses of 2.50, 5, and 10 mg/kgBW for 14 d. Blood glucose, water intake, urine volume, creatinine clearance, renal ratio, and urine density were measured on day 3, 7, and 14. All data were analyzed by two-way ANOVA followed by Duncan Multiple Range Test.

Results: Purified gambir significantly lower blood glucose ($p < 0.05$) and had a significant effect ($p < 0.05$) on renal function parameters in diabetic rats. Duration of treatment purified gambir in diabetic rats had a significant effect ($p < 0.05$) on water intake, urine volume, creatinine clearance and renal ratio.

Conclusion: This result show that purified gambir at dose 2.5 mg/kgBW increased the renal function of diabetic rats, in contrast, purified gambir at dose 5 and 10 mg/kgBW attenuate renal function of diabetic rats

Keywords: Antioxidant, Blood glucose, Purified gambir, Renal function, Creatinine clearance

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INTRODUCTION

Diabetes mellitus (DM) is a chronic condition characterized by hyperglycemia due to the body's inability to produce insulin, lack of insulin or the body's inability to use the insulin produced effectively. In 2019, International Diabetes Federation (IDF) estimated that 463 million or 9.30% of the total population aged between 20-79 y have diabetes. DM is generally considered as multi-factorial and is defined as chronic metabolic disorder which results from damage of pancreatic beta cells or decreased uptake of glucose into cell [1, 2].

Chronic hyperglycemia induces excessive generation of reactive oxygen species (ROS) leading to high level of oxidative stress and is extensively acknowledged as vital component in diabetes induced renal disorders [3, 4]. Chronic hyperglycemia mediated unnecessary generation of ROS is the common factor linking disturbed renal hemodynamics with the dysregulated metabolic pathways [5, 6].

Complications of diabetes have emerged as a global health issue, as well as the leading cause of morbidity and mortality in diabetic patients in the absence of a viable treatment approach. Diabetes is frequently associated with long-term microvascular and macrovascular problems, such as nephropathy, retinopathy and neuropathy [7]. Diabetic nephropathy (DN) is a common microvascular congestion in people with diabetes, shown by proteinuria, which leads to impaired kidney function [8]. Diabetic nephropathy (DN) is a common and a major complication in both type 1 and 2 diabetes mellitus and a prominent cause of end-stage renal disease (ESRD) worldwide.

In recent years, traditional medicines have been growing rapidly to treat diabetes mellitus in several countries, including Indonesia (5). Indonesian people traditionally chewed gambir with dried areca nut and betel leaves. Furthermore, gambier leaves are used in a herbal treatment mixture to cure dysentery, diarrhoea, deafness, spongy gums, and sore throat [9]. Gambier is also listed as a medicinal plant in the Indonesian Medicinal Pharmacopoeia monographs.

Uncaria gambir from the Rubiaceae family has various activities, including antioxidant [10], anti-inflammatory [11], anti-atherosclerosis [12] and hypoglycemic effect. Previous research

revealed that purified gambier reduce blood sugar levels and blood pressure in diabetic-hypertensive rats [13]. However, there is lack information regarding purified gambier effect on renal function in diabetic model. The purpose of this study is to investigate the effect of purified gambier to renal function in diabetic rats.

MATERIALS AND METHODS

Plant materials

Purified gambir been standardized based on Indonesian Herbal Pharmacopoeia standards supplied by Andalas Sitawa Fitolab Ltd. Padang, West Sumatera, Indonesia.

Chemicals

Alloxan, Prednisone, and Glibenclamide were purchased from Merck (Germany), sodium citrate buffer and thiobarbituric acid were obtained from Sigma-Aldrich (USA). All other chemicals used were of analytical grade obtained from commercial sources.

Experiment animals

Male Wistar Kyoto rats weighing 180-220 g were utilised in this investigation. Animals were collected from the Animal House at the Faculty of Pharmacy. All experiments were carried out by the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and the European Council Directive on the Care and Use of Laboratory Animals (86/609/EEC) issued on November 24, 1986, and were approved by the Faculty of Medicine's Research and Ethics Committee (No. 602/UN.16.2/KEP-FK/2022). Before being treated, Wistar Kyoto rats were acclimatized to standard laboratory conditions with 12:12 light/dark cycles for one week and provided standard food and water ad libitum. Animals were kept individually in stainless steel cages (25±2 °C and 40–60% relative humidity).

Diabetic induction

Alloxan 125 mg/kgBW was injected intraperitoneally to rats to develop diabetes. Alloxan was given to the experimental animals after they had been fasted for 16 h. Following alloxan injection, the experimental animals were given a 5% glucose solution for three days. An Accu-check

active glucometer (Roche, Germany) was used to test blood glucose levels in the rat tail. The rats were defined as diabetic after two consecutive measurements of blood glucose above 250 mg/dl and a fasting blood glucose (FBG) level greater than 126 mg/dl [13].

Experimental design

Forty-five diabetic male rats were divided into five groups, each consist of six rats. Diabetic control were treated with vehicle (Na CMC 5%). Glibenclamide control were treated with glibenclamide at the doses of 0.45 mg/kg; the treatment group were treated with Purified Gambir at dose 2.5 mg/kgBW (PG 2.5), dose 5 mg/kgBW (PG 5) and dose 10 mg/kgBW (PG 10).

Blood sugar-lowering activity measurement

The Percentage of fasting blood glucose (FBG) change was measured on before and 14 d after the treatment using the Easy Touch® GCU glucometer. Before that the rats were fasted for ±12 h. Rats were declared diabetes if the results of fasting blood glucose examination were 126 mg/dl.

$$\% \text{ FBG Change} = \frac{\text{FBG day 0 (mg/dl)} - \text{FBG day 14 (mg/dl)}}{\text{FBG day 0 (mg/dl)}} \times 100 \%$$

Water intake measurement

The water intake was measured on day 3, 7 and 14. Water consumption was calculated by subtracting the initial volume of consumption water (125 ml) with the final volume after being given for 24 h.

Urine volume measurement

The urine volume was measured on day 3, 7 and 14. The urine volume of diabetic male rats was measured by placing the rat in a metabolic cage with a plastic urine container. The urine excreted by the rats was then measured after 24 h.

Urine density measurement

The urine density was measured by weighing method using a pycnometer. The urine density measured volume was measured on day 3, 7 and 14.

$$\rho = \frac{\text{pycnometer containing (g)} - \text{empty pycnometer (g)}}{\text{urine volume (ml)}}$$

Creatinine clearance measurement

The creatinine clearance was measured on day 3, 7 and 14. The blood was taken through the orbital sinus of the eye. Measurement of serum creatinine levels was carried out in according to Jaffe method. The serum creatinine levels were measured using Rhiele 5010 V5+spectrophotometer with a wavelength of 492 nm. Urinary creatinine levels were measured from 24 h urine with the same procedure as measuring serum creatinine levels.

$$\text{Creatinine clearance} = \frac{\text{Urine creatinine } (\mu\text{mol/L}) - \text{Urine volume 24 h (ml)}}{\text{Serum creatinine } (\mu\text{mol/L})}$$

Renal weight ratio measurement

The renal weight ratio clearance was measured on day 3, 7 and 14. Measured by weighing the weight of the left and right kidneys. After that it was compared with the body weight of the rat.

$$\text{Kidney Weight Ratio} = \frac{\text{Kidney (g)}}{\text{Body weight(g)}}$$

Data analysis

Data were analysed using two-way ANOVA followed by Duncan Multiple Range Test (DMRT). Significance levels were taken at $p < 0.05$.

RESULTS

Alloxan induced diabetes

In this study, alloxan induction in WKY rats resulted in diabetic condition as shown in table 1. Table 1 shows that within 14 d of induction, fasting blood sugar levels exceed 126 mg/dl. When compared to the period before induction, animals displayed clinical indications that confirmed the results of the measurements, such as

polyuria, polyphagia, polydipsia, balance difficulties, weight loss, and altered behaviour. This study found that alloxan 120 mg/kgBW can induce diabetes in WKY rats.

Purified gambir effect on fasting blood glucose (FBG)

PG displays a significant reduction in fasting blood glucose levels ($p < 0.05$) as shown in table 2. PG 10 has a better effect on lowering blood sugar levels than PG 2.5 and 5, although statistically, PG 10 and 5 are not significantly different ($p < 0.05$). Apart from that, there was a difference in the response to lowering blood sugar between the PG group and the glibenclamide group ($p < 0.05$).

Purified gambir effect on water intake

The results showed that there was no significant effect of dose, duration, and interaction between dose-duration of treatment ($p > 0.10$) on the water intake in diabetic rats. Water intake in all groups remain steady as shown in fig. 1.

Purified gambir effect on urine volume

The results showed that there was a significant effect of the duration of treatment ($p < 0.05$) on the urine volume of diabetic rats. However, the dose variation and interaction between dose duration had no significant effect ($p > 0.10$) on the urine volume in diabetic rats (fig. 2).

Purified gambir effect on urine density

Data showed that there was a significant effect of variation in the duration treatment ($p < 0.05$) on the urine density in diabetic rats as seen in fig. 3. However, interaction between dose-duration had no significant effect ($p > 0.10$) on the urine density in diabetic rats. The average urine density of the diabetes group was higher than another group. before being given the test preparation. Rat given PG experienced an increase in urine density after 3 d, but it decreased until day 14. The difference in PG dosage had no effect on urine density of the test animals.

Purified gambir effect on creatinine clearance

The study showed that there was a significant effect of dose, duration, and interaction between dose-duration treatment ($p > 0.05$) on the creatinine clearance of diabetic rats (fig. 4). All PG group give a lower creatinine clearance compare to glibenclamide control. The PG 2.5 and glibenclamide groups experienced a decrease in creatinine clearance after being given for 3 d; however, on the 7th and 14th d the creatinine clearance value gradually increased, and approached the normal value of creatinine clearance. On the other hand, PG 5 and PG 10 after 3 d experienced an increase in creatinine clearance values, but there was a decrease in creatinine clearance until the 14th d.

Purified gambir effect on renal ratio

The results showed that there was a significant effect of the duration treatment ($p < 0.05$) on the renal ratio in diabetic rats. However, interaction between dose duration had no significant effect ($p > 0.10$) on the renal ratio in diabetic rats (5).

Table 1: Effect alloxan on fasting blood glucose in WKY rats groups

Treatment	Fasting blood glucose (mg/dl)
Baseline	84.3±2.2
Induction	304.1±5.4*

Data are the mean±SE of nine rats, * $p < 0.05$

Table 2: Effect of purified gambir fasting blood glucose change in diabetic rats for 14 d

Treatment group	FBG change (%)±SE
PG 2.5	70.89±2.10 ^a
PG 5	61.00±5.35 ^{bc}
PG 10	60.84±1.90 ^{bc}
Glibenclamide	67.08±2.00 ^{ab}
Diabetic Control	54.95±1.38 ^c

Data are the mean±SE of nine rats, Information: (abc) The average data with different superscripts on columns or rows showed significant differences

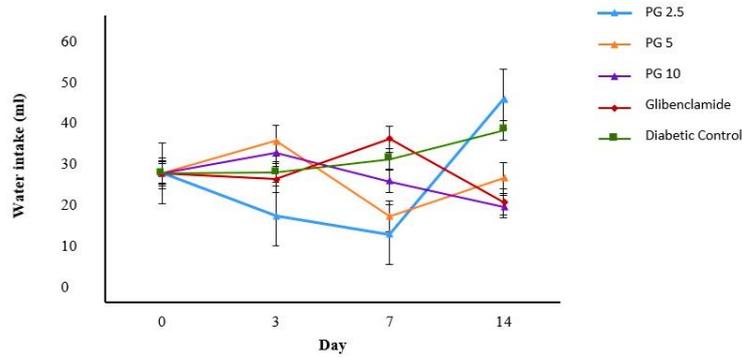


Fig. 1: The effect of purified gambir on water intake in diabetic rats

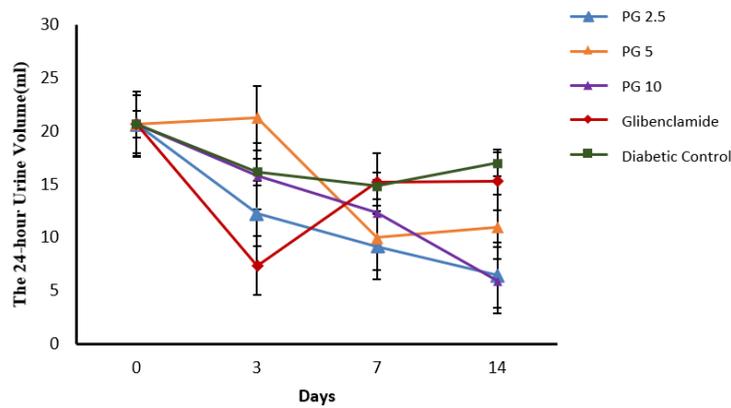


Fig. 2: The effect of purified gambir on the 24 h urine volume in diabetic rats

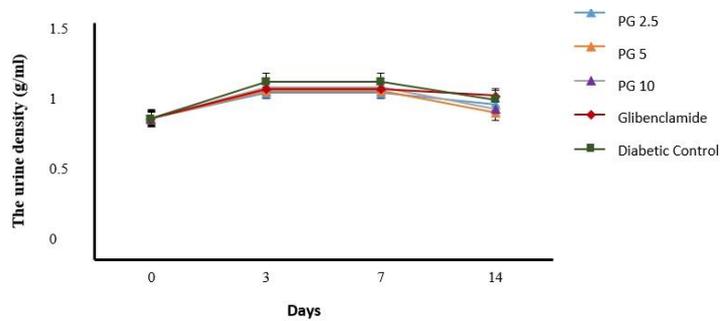


Fig. 3: The effect of purified gambir on the urine density in diabetic rats

Information: (abc) The average data with different superscripts on columns or rows showed significant differences.

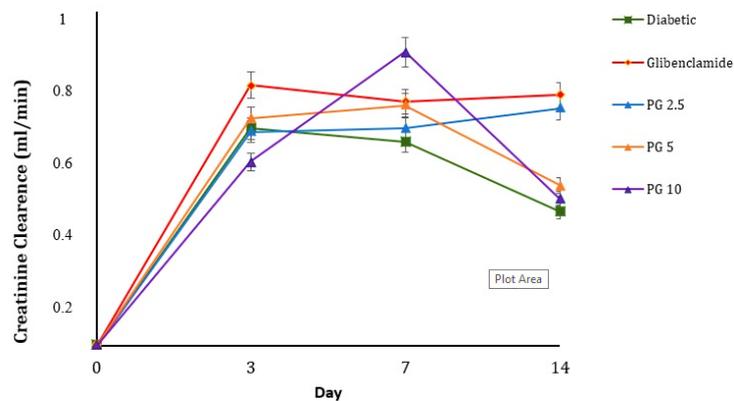


Fig. 4: The effect of purified gambir on creatinine clearance in diabetic rats

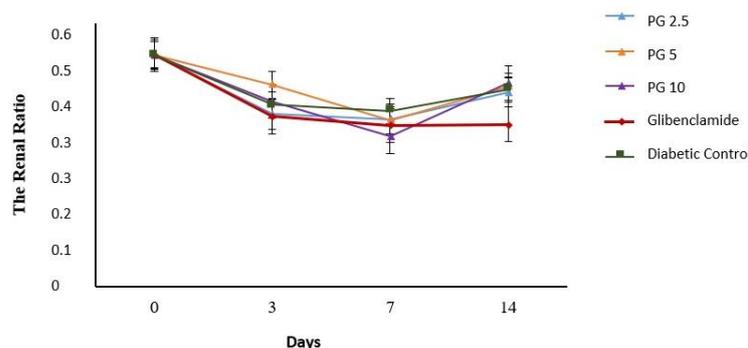


Fig. 5: The effect of purified gambir on the kidney ratio in diabetic rats

DISCUSSION

Hyperglycemia causes the production of free radicals to increase through several molecular pathways, including by increasing cytosolic calcium levels and protein kinase activation. The uncontrolled increase in free radical production accompanied by inadequate antioxidant defence in the body causes oxidative stress conditions [14].

Alloxan-induced diabetic rats cause common symptoms of diabetes in the form of polyuria (increased urine volume), polydipsia (increased thirst) and polyphagia (increased hunger) [15]. The same thing happened in this study, where alloxan-induced diabetic rats had higher urine and drinking volumes than normal rats. The diabetic rats in this study had an average 24 h drinking volume of 27.00 ± 8.66 ml. Meanwhile, according to Castro *et al.*, (2014), Wistar rats have an average 24 h drinking volume of 22.50 ± 7.30 ml [16]. This condition shows one of the manifestations of diabetes in the form of polydipsia. Polydipsia can be caused by several things in the form of acute tubular necrosis, decreased renal perfusion, decreased intravascular volume and decreased blood volume [14].

In this study, the average urine volume in diabetic rats was 20.67 ± 4.69 ml. According to Castro (2014), the average urine volume in normal Wistar rats is 10.90 ± 2.20 ml [16]. Under normal circumstances, 100% of the glucose filtered by the glomerulus is reabsorbed in the renal tubules; however, hyperglycemia in diabetes causes the glucose filtered to surpass the ability of the kidney tubules to resorb. Glucose that cannot be reabsorbed is expelled in the urine, resulting in an osmotic diuretic state that leads to polyuria [17].

Apart from seeing the manifestations of diabetes, drinking volume and urine volume are also useful for seeing the balance of fluid in and out. In this study, fluid imbalance occurred in the PG 2.50 group on the 14th d after administration, in the form of high drinking volume and low urine volume. This can be caused by several things, in the form of acute tubular necrosis, decreased renal perfusion, decreased intravascular volume and decreased blood volume. Apart from that, it can be caused by a high standard error value.

Diabetic rats had an average urine specific gravity of 0.89 ± 0.06 in this study. Meanwhile, rats have an average urine specific gravity of 1.00 ± 0.01 [18], according to Jeffrey (2008). Before being administered PG, diabetic rats showed lower urine specific gravity than normal rats, indicating the development of urine dilution due to increased urine volume [17]. Interestingly, on the third day following medication administration, urine specific gravity increased while urine volume decreased; in addition to urine volume, hyperfiltration conditions indicated by the amount of creatinine clearance on day three influenced urine specific gravity. According to Clark (2019), hyperfiltration conditions increased the specific gravity of rat urine [19].

As previously stated, hyperglycemia in diabetic patients causes increased urine volume, drinking volume, and specific gravity. According to this study, PG administration for 14 d can lower blood sugar levels and diabetic symptoms such as decreased urine volume and specific gravity. This is related to catechins' antioxidant action. In 2021, Sekiou reported that antioxidants can reduce blood sugar

levels and ameliorate diabetes symptoms such as polyuria and polydipsia in alloxan-induced diabetic rats [20].

Catechins' antioxidant activity can lower the quantity of free radicals that induce hyperglycemia via the phenolic hydroxyl group on catechins, contributing one electron to free radicals [21]. Furthermore, catechins have been demonstrated to improve antioxidant enzyme activity in diabetic rats [22]. Apart from antioxidant properties, gambier can lower blood sugar levels by inhibiting the alpha-glucosidase enzyme, which breaks down carbs into glucose that can be absorbed by the blood [23]. Zebua *et al.*, (2018) revealed that gambir has anti-hyperglycemic action comparable to metformin, an anti-diabetic medication that acts by mending damage to pancreatic beta cells [24].

The high value of creatinine clearance in diabetic rats shows the presence of hyperfiltration in the kidneys. Hyperfiltration is more frequent in type 1 diabetes than in type 2 diabetes in the early stages. Diabetes' hyperfiltration process is extremely complicated and regulated by several pathways and mediators [25]. Srivastana (2018) expressed the same point, stating that kidney damage in diabetes begins with a hyperfiltration syndrome characterised by a high glomerular filtration rate [26].

Damage to the kidneys, apart from being seen from the creatinine clearance value, is also seen from the kidney ratio and urine density. Oxidative stress can cause hypertrophy of the kidneys so that the kidneys experience an increase in creatinine clearance, kidney ratio and urine density weight. The hyperfiltration condition that occurred in this study was related to renal clearance. Where the hyperfiltration state is characterized by a high creatinine clearance value followed by a high kidney and urine density ratio, while a decrease in the creatinine clearance value on days 3 and 7 is followed by a decrease in the kidney and urine density ratio.

In this study, PG 5 and 10 experienced hyperfiltration until the third day after administration of the preparation; this is in accordance with previous research by Armenia *et al.*, (2021), which states that gambir at a dose of 5-20 mg can cause hyperfiltration characterised by the percentage value of renal function exceeding 100% [3]. However, this treatment group experienced a decrease in creatinine clearance until day 14. At the same time, the PG 2.5, glibenclamide group, and negative control group experienced a decrease in creatinine clearance after 3 d of administration. This is in accordance with Palatini *et al.*, (2012) who state that hyperfiltration that occurs in the early stages of diabetes, with time will decrease the creatinine clearance value, which will lead to kidney failure [27].

The negative control group in this study showed a decrease in creatinine clearance until day 7. The decrease in creatinine clearance indicated the development of kidney disease in untreated diabetic rats. This is in accordance with a study conducted by Sekiou (2021), which stated that diabetic rats experienced a decrease in creatinine clearance values due to alloxan induction. The reason is that alloxan accumulates in tubular cells that secrete Glucose Transporter 2 (GLUT 2), which causes damage to the glomerulus so that creatinine levels in the blood become high. Creatinine clearance values are low [20]. However, the negative control group on day 14

again experienced hyperfiltration. According to Srivastana (2017), hyperfiltration is one of the adaptive responses due to damage to the kidney glomerulus [26].

Damage to the kidneys, apart from being seen from the value of creatinine clearance, can also be seen from the value of the kidney ratio. In this study, diabetic rats had a kidney ratio of 0.53 ± 0.01 . According to Mahmoodnia (2017), oxidative stress can cause hypertrophy in the kidneys so that the kidneys experience an increase in weight. The kidney ratio was influenced by differences in body weight between treatment groups, which made the interpretation of kidney weight difficult. Supporting data is needed in the form of macroscopic and microscopic data of organs to get more specific results [28].

Hyperfiltration conditions in this study are related to the value of the kidney ratio. Where is the hyperfiltration state on days 0 and 14, which is characterised by a high value of creatinine clearance followed by a high value of the kidney ratio, while a decrease follows a decrease in creatinine clearance value on days 3 and 7 in the value of the kidney ratio. This is in accordance with Chagnag's (2018) statement, which states that hyperfiltration conditions cause hypertrophy of the kidneys [29].

The result of six parameters showed an effect of diabetes on urine volume in the form of polyuria, 24 h drinking volume in the form of polydipsia, increased urine specific gravity, decreased kidney ratio and creatinine clearance, which indicated a decrease in kidney function. PG administration can reduce blood sugar levels and overcome the manifestations of diabetes in the form of polyuria, reduce urine specific gravity and increase kidney ratio. However, the condition of polydipsia still persists. Furthermore, purified gambier in small doses can improve kidney function and lower blood sugar levels. This can be seen from the value of creatinine clearance, which increased to close to normal values until the end of the experiment after experiencing a decrease due to kidney disorders and related to previous research [13].

At the same time, renal function decreased until the end of the experiment after hyperfiltration occurred. This is because the use of Gambier in large doses is a pro-oxidant. It should be with the interpretation of the results and their comparison with those of other studies. There is no need to repeat the results, review literature and textbook knowledge, or cite references that do not have a close relationship with the present result.

CONCLUSION

In summary, the antioxidant activity of PG reduces fasting blood glucose levels in alloxan-induced diabetic rats. This result show that PG at dose 2.5 mg/kgBW increased renal function of diabetic rats, in contrast, PG at dose 5 and 10 mg/kgBW attenuate renal function of diabetic rats.

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AUTHORS CONTRIBUTIONS

All authors contributed to the manuscript and approved the submitted version

CONFLICT OF INTERESTS

All authors declare that there is no conflict of interest regarding the publication of this article.

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