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# The Effectiveness of Cardamom Seed Extract (*Amomum compactum*) Against Urea Creatinine and Histopathology Obese Rat Kidney

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**ABSTRACT**

There are several factors that can cause kidney damage, one of which is obesity. Obesity itself can cause complex metabolic disorders that can affect kidney physiology. The biological activity of flavonoid components in *A. compactum* showed that cardamom extract had high anti-inflammatory and antioxidant activity. This study was a true experimental with post tests only of control group design and was conducted from July to August 2022 at Pusat Study Pangan dan Gizi (PSPG), Gadjah Mada University. A total of 30 Wistar rats were used in this study and which randomly divided into normal control, negative control, 45 mg dose cardamom, 90 mg dose cardamom, 180 mg dose cardamom groups. Obesity was obtained by high fat and high carbo diet. At the end of the study, the rats will be terminated and urea, creatinine was examined, renal histopathology changes were observed using hematoxylin and eosin strain. There was differences in urea levels between groups K and P1, P2, P3; P1 and P2, P1 and P3, and P2 and P3 ($p<0.005$). There was differences in creatinine levels between groups K and P1, P2, P3; P1 and P2, and P1 and P3 ($p<0.005$). There was a significant difference in renal histology between groups normal control, negative control, and P1 ($p<0.001$). Cardamom seed extract is effective in reducing urea creatinine levels and improving histopathology obese rat kidney.



## 1. INTRODUCTION

Kidneys are organs of the body that have a function to filter and remove waste substances from the body's metabolism from the blood and maintain fluid and electrolyte balance. On the other hand, the kidney is also an organ that is susceptible to the influence of chemicals, because this organ receives 25-30% of the blood circulation to be cleaned, so as a filtration and excretory organ the possibility of pathological changes is very high. An increase in the excreted substances can cause kidney damage due to poisoning caused by contact

with these materials. This kidney tissue damage if left unchecked can lead to a progressive decline in kidney function which is characterized by an increase in metabolic products such as urea and creatinine and generally ends in kidney failure [1].

There are several factors that can cause kidney damage, one of which is obesity. Obesity itself can cause complex metabolic disorders that can affect kidney physiology. The World Health Organization (WHO) states that obesity has become a world epidemic problem that causes the second most deaths after smoking. According to a 2018 WHO survey, 97 million adults in the United States are obese. In Indonesia, the high incidence of obesity in recent years is evidenced by the 2018 Riskesda survey which states that there is a tendency to increase the number of obese people, both men and women. This condition is substantially implicated in an increased risk of diabetes, cardiovascular disease, and chronic kidney disease (CKD) [2], [3].

Cardamom (*A. compactum*) is an herbal spice that is often used in culinary and traditional medicine practices. *A. compactum* is native to Indonesia and grows wild in the forests on the island of Java, but is now widely cultivated in various regions in Indonesia. Central Java, East Java, West Java, and West Sumatra are some of the *A. compactum* producing areas. Indonesian people use *A. compactum* for various purposes such as cooking spices, health drinks, traditional medicines, and aromatherapy. Besides being used as a cooking spice, *A. compactum* is also used as a medicinal ingredient [4]. Various kinds of bioactive components can provide a synergistic effect that is beneficial to the body. The active substance in *A. compactum* can improve the adverse effects of metabolic syndrome including dyslipidemia that occurs in obese patients, namely by increasing the rate of cholesterol degradation, activating lipoprotein lipase, reducing lipid absorption from the intestine [4]. In addition, the biological activity of flavonoid components in *A. compactum* showed that cardamom extract had high anti-inflammatory and antioxidant activity [5].

Based on the above background and the absence of further research on the effectiveness of cardamom seed extract (*A. compactum*) to improve kidney function due to obesity, the authors wanted to find out more about the effectiveness of cardamom seed extract (*A. compactum*) against urea creatinine and renal histopathology of obese rats. This study is expected to provide useful results considering the high prevalence of impaired kidney function due to obesity that many people suffer from. This study aims to examine the relationship between administration of cardamom seed extract with urea creatinine levels and histopathological picture of rat kidney.

## 2. METHOD

This study was a true experimental with post tests only of control group design and was carried out at Pusat Study Pangan dan Gizi (PSPG), GADJAH MADA University in July-August 2022 using 30 Wistar rats of 6-8 weeks and weighed 250 ± 50 g. The Ethics Committee of the Faculty of Medicine, Diponegoro University approved this study (No:73/EC/H/FK-UNDIP/VII/2022). The sample was calculated using the Federer method regarding the concept of reduction, replacement, and refinement. The rats were randomly divided into five groups, namely the normal control, negative control, 45 mg dose cardamom, 90 mg dose cardamom, 180 mg dose cardamom groups. Data were analyzed using SPSS for Windows software.

### *2.1 Animal Treatment*

Thirty Wistar male rats (6-8 weeks, 250 ± 50 g) were obtained from GADJAH MADA University, Yogyakarta, housed in a controlled environment and provided with standard rodent chow and water ad libitum. The obesity was induced by High Carbohydrate Diet (HCD) and High Fat Diet (HFD) for 30 days. Wistar rats were divided into five groups of treatment, consisting of 2 control groups and 3 treatment groups.

Normal control group were given standard rodent chow and water. Negative control group were obesity induced by HCD and HFD for 30 days. Treatment groups were obesity induced and 45 mg, 90 mg, and 180 mg/kg BW/days cardamom for 14 days. On day fifteenth, venous blood was taken, then the rats were terminated to collect renal histopathology sample.

### *2.2 Renal Histopathology Assessment*

Renal specimens were prepared for histopathological assessment to examine renal tissue damage using hematoxylin and eosin stain. Renal proximal tubular damage of Wistar rats was examined by calculating the lumen of the closed proximal tubule and necrosis of the proximal tubule in 5 fields of view for each Wistar rat in each group using 300x magnification on binocular microscope.

## 3. RESULTS

Thirty Wistar rats were used as samples in this study. Rats were divided into 5 different groups using simple random sampling methods. Groups consisted of 2 control groups and 3 treatment groups. The rats were obese, the obesity was induced by HFD and HCD, and given oral cardamom extract. All Wistar rats were survived until the end of study and terminated to examine blood urea creatinine level and renal histopathology.

### *3.1 Creatinine and Urea Level*

The mean urea and creatinine level after high fat diet and treatment with cardamom extract and the results of the normality test shows that urea level doesn't have normal distribution in all groups. Meanwhile, creatinine level was normally distributed in all groups. (Table 1) These results indicated that data analysis of creatinine level will be continued using parametric test, while urea level will be tested using non parametric test.

Based on ANOVA results, it is shown that there's a significantly different between groups in creatine levels in this study. The analysis was carried out using Games Howell test to analyze creatinine level differentiation between groups in this study. The results showed that there is a significantly different between normal control, negative control, treatment group 1 in this study, indicated with p values less than 0,005. While treatment group 2 and 3 showed no significant differentiation from statistical analysis. (Table 2)

Kruskal Wallis test was carried to analyze the differentiation within urea level in this study groups. The results showed $p < 0,005$ indicated that urea levels was significantly different between groups. Data analysis was continued using Mann Whitney test, the results showed there is a significantly different between groups in terms of urea level in this study. (Table 2)

### *3.2 Renal Histopathology*

From the histopathological data obtained, the rat kidney was tested for level of agreement using the intra-class correlation coefficient (ICC) test, the results were 0.999 and p value was 0.000, indicating a very good agreement between the two examiners. (ICC 0.999 with confidential interval (95%: 0.998 – 1000). (Table 3)

From the data obtained, then a normality test was performed using the SAPHIRO-WILK. Based on the normality test using SAPHIRO-WILK showed that the results of the kidney histopathology of the tested rats were not all $p > 0.05$, it can be concluded that the data were not normally distributed. (Table 3)

From KRUSKAL-WALLIS test, it was found that $p < 0.05$, so it can be concluded that the renal histopathology data in the five groups were significantly different. (Table 3) Then data was assessed the differences in the kidney histopathology of rats between groups. Data analysis was continued using Mann Whitney test. There were significant differences between the normal control group and the negative control

group, treatment group 1 (P1) research ($p<0.05$), normal control group and treatment group 2 (P2), treatment group 3 (P3), no significant difference was found. ($p>0.05$). (Table 4)

## 4. DISCUSSION

In the study of [6] showed that giving a high-fat diet in rats, ectopic lipid accumulation and increased fat deposition in the renal sinuses, can also cause hypertension in the glomerulus and increase glomerular permeability due to damage to the glomerular filtration barrier due to hyperfiltration resulting in glomerulomegaly and focal segmental glomerulosclerosis. This incidence is often referred to as obesity-related glomerulopathy (ORG). ORG often appears together with pathophysiological processes associated with other comorbidities or the elderly, which contributes to more severe kidney damage in obese patients. In a study conducted by Verma, it was found that the activity of the free radical scavenger contained in cardamom seeds in the form of 1,8-cineol oil, -terpinol, protocatechualdehyde and protocatechuic acid which have potential health benefits by inhibiting lipid peroxidation, thereby improving the histopathology of damaged rat organs. normal control group and treatment group (which received cardamom). This is consistent with this study, namely the results of the negative control group with HFD and HDC without cardamom administration had higher urea and creatinine values and the percentage of kidney histopathological damage in rats was higher than the group given cardamom. Cardamom administration as anti-obesity, antioxidant, and anti-inflammatory can stabilize urea and creatinine levels, reduce oxidative stress due to ectopic fat accumulation in the renal sinuses by inhibiting lipid absorption through the activation of lipid-breaking enzymes (lipoprotein lipase), in addition to the anti-inflammatory effect of cardamom. able to suppress kidney damage by inhibiting inflammatory mediators such as cytokines, interleukins and prostaglandins, so that the increase in urea and creatinine levels in rats receiving HFD and HCD can be suppressed and tends to be more stable than the group that only received HFD and HCD without cardamom. So, it can be concluded that cardamom administration has an effect on urea and creatinine levels in mice that have been given HFD and HCD. However, the non-significant difference between groups P2 and P3 showed that the level of urea and creatinine in rats given HFD and HCD and had received cardamom extract was not affected by the dose of cardamom given.

This conclusion is in accordance with research conducted by Rahman et al who concluded that administration of HFD and HCD can increase kidney cell damage which affects the increase in kidney markers (ureum and creatinine) [7]. HFD and HCD trigger the formation of free radicals in the body externally, which can affect against kidney cells. Administration of HFD and HCD for 30 days has been shown to cause impaired kidney function. The mechanism of HFD and HCD as inducers of oxidative stress can be explained through the resulting oxygen radicals and hydrogen peroxide. The production of these oxidant agents further causes oxidative DNA damage and cell membrane peroxidation. Damage to DNA and cell membranes is what affects kidney function to the death of kidney cells [8].

In addition to urea and creatinine levels, this study also investigated the administration of cardamom to the kidney histopathology of rats that had been given HFD and HCD. According to the results of histopathological research, it was analyzed by two observers. From the results of these two observations, it was found that the administration of cardamom to rats given HFD and HCD had histopathological improvement similar to that of rats in the normal control group, which only received standard feed without HDF and HCD and cardamom.

These results are in accordance with research conducted by Bhaswant M, et al regarding the effect of cardamom on kidney damage caused by not by the metabolic syndrome. In this study, it was explained that rats given cardamom had a decrease in visceral adiposity, total body fat mass, systolic blood pressure and plasma triglycerides, as well as organ structure, compared to those not given cardamom. Contains flavonoids

which are included in the polyphenol group which is thought to be able to increase the effect of antioxidants in the body. The activity of free radical scavengers contained in cardamom (*A. compactum*) in the form of 1,8-cineol oil, -terpinol, protocatechualdehyde and protocatechuic acid which have potential health benefits by inhibiting lipid peroxidation. Inhibition of lipid peroxidase has a positive impact on the body, namely increasing the number of endogenous antioxidants in the body [9]. Directly 1,8-cineol oil, -terpinol, protocatechualdehyde and protocatechuic acid will donate hydrogen atoms (H+) to neutralize free radicals in the body. cell. The mechanism of action of flavonoids (FlOH) as antioxidants is by transferring hydrogen atoms (H) from the hydroxyl group (OH) to free radicals so that flavonoids turn into flavonoid phenoxy. The flavonoid phenoxy radicals formed will be attacked again by free radicals to form a second flavonoid phenoxy radical. This is because the flavonoid phenoxy radical has a conjugated double bond, so it can balance it by electron delocalization so that it becomes a stable quinone compound. The presence of flavonoids helps neutralize free radicals so that a positive effect is obtained in cells [4]. When free radicals are controlled, this will prevent the occurrence of lipid peroxidation in the ER and mitochondria due to ROS. A stable state allows cells to make repairs automatically to return to their original function. When the situation has returned to normal, the activity of the ER and mitochondria will return to normal. Mitochondria that function to produce SOD (superoxide dismutase) are able to produce SOD again. SOD can work normally to neutralize ROS generated by oxidative metabolism of cells under normal conditions [8].

The active compounds in cardamom such as alkaloids, flavonoids, terpenoids and tannins. Flavonoid compounds can inhibit enzymes that play a role in the synthesis of prostaglandins. Triterpenoids as anti-inflammatory can reduce cells expressing induced nitric acid synthase (INOS), for example lupeol or by inhibiting nitric oxide production by reducing INOS expression. Several studies have shown the effect of ethanol extract from Java cardamom on several inhibitors such as inhibiting the production of nitrate (NO), prostaglandin E2 (PGE2), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), inhibiting the expression protein from nitric oxide synthase that can be induced, inhibits cyclooxygenase-2, and inhibits nuclear factor-κB (NF-κB) translocation so that the process of cell damage can be suppressed [10].

## 5. CONCLUSION

Cardamom seed extract is effective in reducing urea creatinine levels and improving histopathology obese rat kidney

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## 6. REFERENCES

[1] GJ T, BH D. Principles of anatomy and physiology. John Wiley & Sons; 2018.

[2] Anwar F, Abbas A, Alkharfy K. Cardamom (Amomum compactum Maton) Oils. In: Essential Oils in Food Preservation, Flavor, and Safety [Internet]. 2015. p. 37–72. Available from: https://www.researchgate.net/publication/269107473_What_is_governance/link/548173090cf22525dcb61443/download%0Ahttp://www.econ.upf.edu/~reynal/Civil wars_12December2010.pdf%0Ahttps://think-asia.org/handle/11540/8282%0Ahttps://www.jstor.org/stable/41857625

[3] Mills KT, Xu Y, Zhang W, Bundy JD, Chen C-S, Kelly TN, et al. A systematic analysis of worldwide population-based data on the global burden of chronic kidney disease in 2010. Kidney Int. 2015 Nov;88(5):950–7.

[4] Yahyazadeh R, Rahbardar MG, Razavi BM, Karimi G, Hosseinzadeh H. The effect of elettaria cardamomum (cardamom) on the metabolic syndrome: narrative review. Iran J Basic Med Sci. 2021;24(11):1462–9.

[5] Silalahi M. Bioaktivitas Amomum compactum Soland ex Maton dan Perspektif Konservasinya. J Pro-Life. 2017;4(2):320–8.

[6] Fatemeh Y, Siassi F, Rahimi A, Koohdani F, Doostan F, Qorbani M, et al. The effect of cardamom supplementation on serum lipids, glycemic indices and blood pressure in overweight and obese pre-diabetic women: A randomized controlled trial. J Diabetes Metab Disord. 2017;16(1):1–9.

[7] Alia F, Putri M, Anggraeni N, Syamsunarno MRAA. The Potency of Moringa oleifera Lam. as Protective Agent in Cardiac Damage and Vascular Dysfunction. Front Pharmacol. 2022;12(January):1–18.

[8] Rachmatulloh BN. Pengaruh Terapi Ektrak Kapulaga Hijau (Elettaria Cardamom) Terhadap Aktivitas Enzim SOD (Superoksida Dismutase) dan Histopatologi Hepar Pada Tikus Wistar (Rattus Norvegicus) Model Steatosis Hasil Induksi CCl4. [Malang]; 2017.

[9] Nasution SH, Syarif S, Musyabiq S. Penyakit Gagal Ginjal Kronis Stadium 5 Berdasarkan Determinan Umur , Jenis Kelamin , dan Diagnosa Etiologi di Indonesia Tahun 2018 Chronic Kidney Failure Disease Stage 5 Based on Determinants of Age , Gender , and Diagnosis of Etiology in Indonesia in 201. JK Unila. 2020;4(2):157–60.

[10] Owolabi OO, James DB, Sani I, Andongma BT, Fasanya OO, Kure B. Phytochemical analysis, antioxidant and anti-inflammatory potential of Feretia apodanthera root bark extracts. BMC Complement Altern Med. 2018;18(1):1–9.

**Table 1.** Creatinine-Urea Level Analysis

| No | Groups | Substance Level | | | | | |
|---|---|---|---|---|---|---|---|
| | | Urea (mg/dL) | Uji Normalitas | P | Creatinine (mg/dL) | Uji Normalitas | P |
| 1 | Normal Group/ C (+) | 10,53 | .034[s] | 0,000[k] | 0,75 | .535[s]* | 0,000[§] |
| | | 10,23 | | | 0,76 | | |
| | | 10,82 | | | 0,72 | | |
| | | 10,23 | | | 0,75 | | |
| | | 11,70 | | | 0,78 | | |
| | | 10,23 | | | 0,76 | | |
| 2 | Negative Control Group/ C (-) | 42,69 | .657[s]* | | 3,07 | .990[s]* | |
| | | 42,69 | | | 3,22 | | |
| | | 44,74 | | | 3,27 | | |
| | | 42,11 | | | 3,19 | | |
| | | 40,94 | | | 3,15 | | |
| | | 42,98 | | | 3,14 | | |
| 3 | Treatment I/ T I | 24,56 | .502[s]* | | 1,56 | .650[s]* | |
| | | 22,22 | | | 1,72 | | |
| | | 24,56 | | | 1,67 | | |
| | | 21,35 | | | 1,63 | | |
| | | 20,47 | | | 1,74 | | |
| | | 25,73 | | | 1,46 | | |
| 4 | Treatment II/ T II | 16,67 | .243[s]* | | 1,15 | .830[s]* | |
| | | 15,79 | | | 1,11 | | |
| | | 14,91 | | | 1,02 | | |
| | | 16,96 | | | 1,27 | | |

| | | | | | |
|---|---|---|---|---|---|
| | | 16,37 | | 1,14 | |
| | | 16,96 | | 1,17 | |
| 5 | Treatment III/ T III | 13,45 | .110[s]* | 1,11 | .990[s]* |
| | | 13,16 | | 1,02 | |
| | | 12,87 | | 1,07 | |
| | | 13,45 | | 1,09 | |
| | | 13,45 | | 1,04 | |
| | | 12,57 | | 1,06 | |

Note: [§]ANOVA; [k]Kruskal-Wallis; *Significant; [s]Saphiro-Wilk

**Table 2.** Between groups comparison on Urea-Creatinine Level

| Groups | Compared groups | Urea | Creatinine |
|---|---|---|---|
| C (+) | C (-) | 0.002[b*] | 0.000[g*] |
| | T I | 0.002[b*] | 0.000[g*] |
| | T II | 0.002[b*] | 0.000[g*] |
| | T III | 0.002[b*] | 0.000[g*] |
| C (-) | T I | 0.002[b*] | 0.000[g*] |
| | T II | 0.002[b*] | 0.000[g*] |
| | T III | 0.002[b*] | 0.000[g*] |
| T I | T II | 0.002[b*] | 0.000[g*] |
| | T III | 0.002[b*] | 0.000[g*] |
| T II | T III | 0.002[b*] | 0.290[g] |

Note: [b]Mann-Whitney; [g]Games-Howell

**Table 3.** Renal Histopathology Analysis

| No | Observer 1** | | Observer 2** | | Normality Test | P |
|---|---|---|---|---|---|---|
| | Groups | % Damage/ slide* | Groups | % Damage/ slide | | |
| | | | | | .591[s*] | .001[k*] |
| 1 | C (+) | 7,35 | C (+) | 11,89 | | |
| 2 | | 22,73 | | 22,59 | | |
| 3 | | 23,83 | | 20,98 | | |
| 4 | | 29,59 | | 31,36 | | |
| 5 | | 49,14 | | 49,54 | | |
| 6 | | 19,81 | | 15,05 | | |
| 7 | C (-) | 30,01 | C (-) | 30,02 | .027[s] | |
| 8 | | 33,32 | | 32,45 | | |
| 9 | | 36,38 | | 36,00 | | |
| 10 | | 90,30 | | 89,22 | | |
| 11 | | 92,43 | | 91,38 | | |
| 12 | | 97,29 | | 94,49 | | |
| 13 | T I | 48,67 | T I | 50,94 | .597[s*] | |
| 14 | | 40,76 | | 39,28 | | |
| 15 | | 24,55 | | 22,37 | | |
| 16 | | 47,38 | | 47,96 | | |
| 17 | | 60,49 | | 61,79 | | |
| 18 | | 57,37 | | 60,33 | | |
| 19 | T II | 12,54 | T II | 13,27 | .104[s*] | |
| 20 | | 23,58 | | 16,37 | | |
| 21 | | 18,53 | | 16,99 | | |
| 22 | | 23,17 | | 13,11 | | |
| 23 | | 22,09 | | 21,76 | | |
| 24 | | 12,67 | | 11,01 | | |

| | | | | | |
|---|---|---|---|---|---|
| 25 | T III | 26,41 | T III | 22,70 | .376[s*] |
| 26 | | 28,55 | | 26,24 | |
| 27 | | 11,35 | | 10,48 | |
| 28 | | 17,42 | | 16,62 | |
| 29 | | 6,50 | | 6,67 | |
| 30 | | 5,19 | | 6,11 | |

Note: [k]Kruskal-Wallis; *Significant; [s]Saphiro-Wilk

**Table 4.** Between groups comparison on Renal Histopathology

| Groups | Compared groups | Sig |
|---|---|---|
| C (+) | C (-) | .015[b] |
| | T I | .041[b] |
| | T II | .310[b] |
| | T III | .240[b] |
| C (-) | T I | .699[b] |
| | T II | .002[b*] |
| | T III | .002[b*] |
| T I | T II | .002[b*] |
| | T III | .009[b*] |
| T II | T III | .589[b] |

Note: [b]Mann-Whitney; *Significant

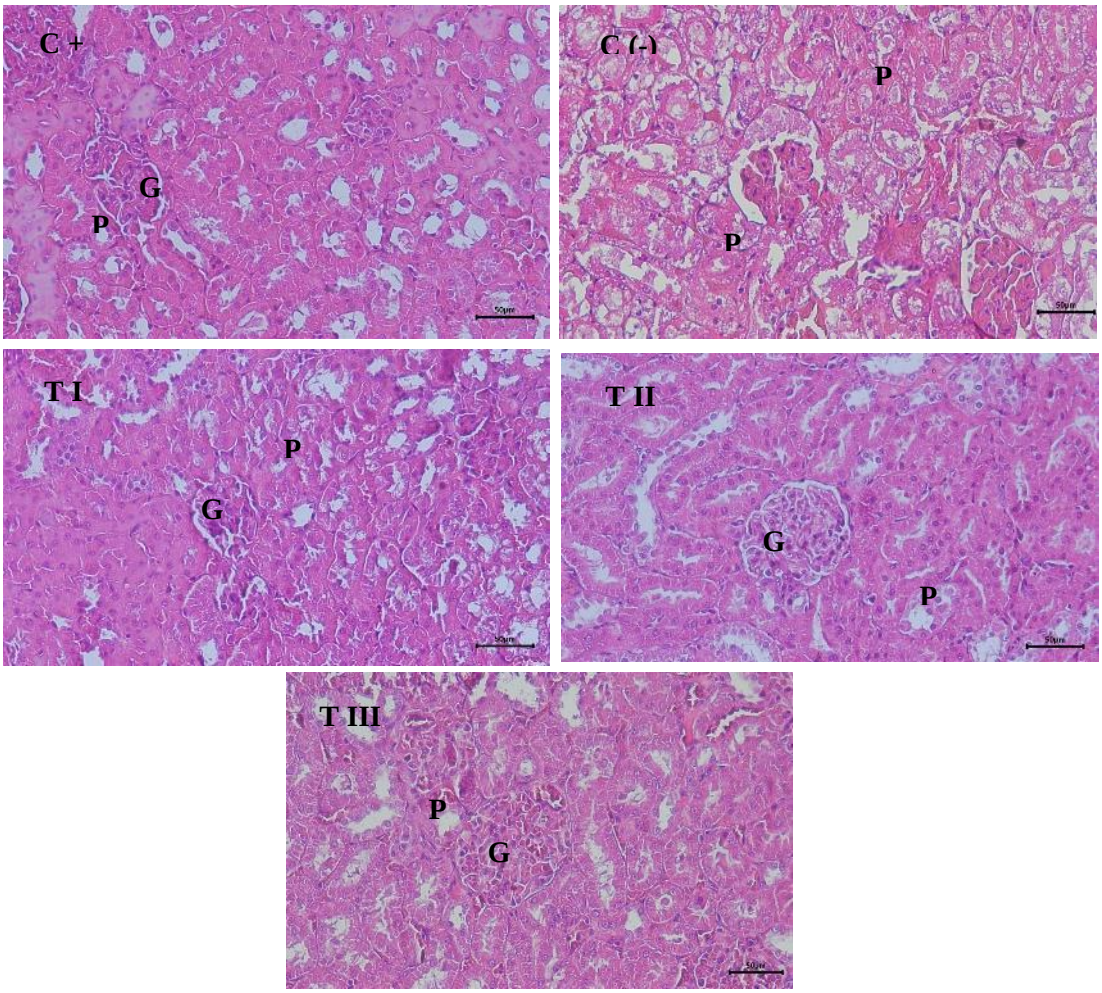


**Figure 1.** Renal Histopathology