The Gastroprotective Role of Yellow Kepok Banana (Musa x Paradisiaca L . va r. Kepok) Peel Extract and Influence on Markers of Oxidative Stress: Malondialdehyde and Nitric Oxide

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ORIGINAL ARTICLE

The Gastroprotective Role of Yellow Kepok Banana (*Musa* x *Paradisiaca* L. var. *Kepok*) Peel Extract and Influence on Markers of Oxidative Stress: Malondialdehyde and Nitric Oxide

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Abstract

Background/Aim: Flavonoids, tannins, saponins and polyphenols in yellow kepok banana (*Musa* x *paradisiaca* L. var. *kepok*) peel potentially could be a solution for peptic ulcer prevention. This study aimed to prove the efficacy of kepok banana peel extract as gastroprotective by analysing the number of gastric ulcers and markers of oxidative stress - malondialdehyde (MDA) and nitric oxide (NO).

Methods: The study was performed on 33 female Wistar rats aged 3-4 months, weighed 100-250 g. Rats were divided into 3 groups: Musa Paradisiaca Var Kepok 1 (MPVK1) treatment group, Musa Paradisiaca Var Kepok 2 (MPVK2) and control group (K). In MPVK1 kepok banana peel extract at a dose of 80 mg / 200 g body weight (BW) was given and the MPVK2 group dose was 160 mg / 200 g BW. The gastritis induction was performed by using 5 % acetylsalicylic acid at a dose of 1500 mg/kg BW. MDA examination by HPLC method, NO examination by ELISA method and macroscopic examination by counting the number of ulcers on the gastric mucosa was performed.

Results: The results showed that the lowest average MDA level, as well as the highest average NO level was in the MPVK2 group 3.27 and 286.17, respectively. The highest mean number of ulcers was in the control group 3.55. By analysing all the results it can be concluded that there is a significant difference in the average levels of MDA ($p = 0.013$), NO ($p < 0.001$) and the number of ulcers ($p < 0.001$) in the three groups.

Conclusion: Banana peel extract was proven to be effective as a gastroprotective through markers of MDA, NO and the number of ulcers in Wistar rats.

Key words: Banana peel; Malondialdehyde; Nitric oxide; Gastroprotection.

Introduction

According to World Health Organization (WHO), the occurrence of gastritis in Indonesia is 40.8 % with quite high incidence rate in several regions in Indonesia, with a prevalence of 274,396 cases out of 238,452,952 people. Gastritis is one of the 10 most common diseases in hospitals with 33,154 cases (4.9 %).[1, 2]

Gastritis is caused by hypersecretion of hydrochloric acid and pepsin which erode the lining of

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the gastrointestinal mucosa.[3, 4] When the stomach is exposed to gastric mucosal destroying agents (acetylsalicylic acid) there will be a back diffusion of H^+ from the lumen into the mucosa, causing a reaction that can harm the stomach and release a large amount of pepsin. Many sodium (Na^+) and plasma proteins enter the lumen and release histamine.[5, 6] This results escalation in secretion of hydrochloric acid by parietal cells, increased capillary permeability and bleeding. In addition, it stimulate local parasympathetic system due to the increase of hydrochloric acid secretion so that venous congestion gets worse and eventually causes bleeding. If this condition is admissible to continue, superficial erosion or ulceration may occur. The process of gastritis is due to an imbalance between mucosal defences and several aggressive factors. One of them is caused by long-term consumption of non-steroid anti-inflammatory drugs (NSAIDs). The exogenous aggressive factors that cause deterioration to the gastric mucosa are in the form of inflammation, or if it is chronic inflammation it can cause bleeding and perforation.[7, 8]

The popular way to prevent the formation of peptic ulcers is by administering drugs that function as cytoprotective on the gastric mucosa. Hence, prevention efforts that have minimal side effects are needed, including the use of tropical plants in the development of phytopharmaceutical use, considering that Indonesia is rich in a variety of medicinal plants.[9–11] Flavonoid antioxidants, tannins, saponins and polyphenols have benefits as anti-inflammatory and antioxidant in hyper lipidemic DM rabbits.[12, 13]

Further, banana peel extract contains flavonoids, tannins, saponins and polyphenols. Tannins minimise gastric acid secretion and have a cytoprotective effect.[9, 11] Tannins also promote tissue reconstruction, inhibit gastric acid production, act as antioxidants and inhibit the activity of *Helicobacter pylori*. Saponins inhibit gastric acid production and lower the pH levels of gastric juices.[14] Based on this, the banana peel has potential as a gastroprotector.[15, 16] This study investigated the extract of the yellow kepok banana (*Musa paradisiaca L. var Kepok*) peel as a gastroprotective in acetylsalicylic acid-induced Wistar rats. Thus, the aim of this study was to prove the effect of kepok banana peel extract on gastritis through markers of oxidative stress (malondialdehyde (MDA), nitric oxide - NO) and the number of ulcers.

Methods

The preparation of kepok banana peel extract

The kepok banana (*Musa* x *paradisiaca* L. var. *kepok*) peel extract was prepared to refer to the banana peel extraction procedure (Copyright Document No HKI S00201809745) initiated with the preparation of the raw materials.

Extract characteristics

Kepok banana is one of the banana varieties in Indonesia. Kepok bananas consist of white kepok bananas and yellow kepok bananas. The part used to be extracted in this study is the peels. The chopped banana peels were dried in an oven at 40 °C for 24 h until they were completely dry, characterised by a texture that was easily broken by hand squeezing. Extract was provided in two doses, namely kepok banana peel extract at a dose of 80 mg / 200 g body weight (BW) with 0.3 % Sodium carboxymethyl cellulose (NaCMC) solvent, 160 mg / 200 g BW yellow kepok banana peel extract with 0.3 % NaCMC solvent and control with 0.3 % NaCMC.

Phytochemical screening extract procedures

The banana peel was washed, roughly sliced and then processed to dry by aerating. The drying process was carried out using an oven and further mashed. The extraction method used 76 % ethanol maceration.

Experimental animals

The experimental animals used in this study were Wistar rats kept in group cages sized 20 x 33 cm in the Experimental Animal Laboratory of Universitas Muhammadiyah Semarang, Indonesia. The environmental conditions of the cage were arranged at 24-26 ^{0}C, supported by sufficient ventilation. The food was provided in the form of pellets, while its drinking water was provided *ad libitum* in the cannula bottles.

Experimental model

The experimental animal samples consisted of 33 female Wistar rats aged 3-4 months weighed 100-250 g. The gastritis experimental animal model was prepared by induction using 5 % acetylsalicylic acid at a dose of 1500 mg/kg BW in one-time administration.[7]

This research was conducted for 18 days. Data collection were in the form of blood serum and counting the number of ulcers and it was carried out on the 18th day (post-test). The study began with the rats being adapted for 14 days. On the

15th day, the rats were divided into 3 groups: Musa Paradica Var Kepok 1 (MPVK1) treatment group, Musa Paradica Var Kapok 2 (MPVK2) treatment group and control group (K). The groups were divided using a random sampling technique. Treatment group 1 (MPVK1) rats were given the yellow kepok banana peel extract at a dose of 80 mg / 200 g BW with 0.3 % NaCMC solvent and treatment group 2 (MPVK2) rats were given 160 mg / 200 g BW yellow kepok banana peel extract with 0.3 % NaCMC solvent. The control group (K) was given 0.3 % NaCMC. On the 16th day, the rats were enforced to not eat for 24 h while still being given water *ad libitum*. On the 17th day, the rats in group K were given 0.3 % NaCMC, meanwhile MPVK1 group was given the yellow kepok banana peel extract at a dose of 80 mg / 200 g BW with 0.3 % NaCMC solvent and the MPVK2 group was given a yellow kepok banana peel extract at a dose of 160 mg / 200 g BW with 0.3 % NaCMC solvent. After one hour, all rats were induced with 5 % asetylsalicylic acid at a dose of 1500 mg/kg BW. On the 18th day, the rats were anesthetised and blood was taken through the orbital sinus, to be terminated. The stomach organs were taken and washed with 0.9 % NaCl and the number of ulcers was counted. The blood serum preparations were examined for markers of oxidative stress (MDA, NO). The MDA examination by high-performance liquid chromatography (HPLC) method, NO examination by enzyme-linked immunosorbent assay (ELISA) method and the macroscopic examination by counting the number of ulcers on the gastric mucosa were performed.

Data processing

The normality of data distribution was tested and appropriate tests were used: One-way ANOVA for data with normal distribution and Kruskal-Wallis for abnormal data. The limit of the degree of significance was set at $p < 0.05$ with 80 % research power and 95 % confidence interval.

Ethical Consideration

The study was successfully accepted by Gadjah Mada University, Indonesia review board with institutional review board (IRB) decision No 00017/LPPT/VI/2021.

Results

Sample characteristics

The samples consisted of 33 female Wistar rats aged 3-4 months weighed 100-250 g. They were grouped into MPVK1, MPVK2 and K group, the minimum weight of rats in the MPVK1 group was 170, the maximum weight was 250 while the average was 207. The minimum body weight for rats in the MPVK2 group was 180, the maximum was 250 and the average was 217. At the same time, the minimum body weight for the rats in the control group (K) was 178, the maximum was 250 and the average was 210, as shown in Figure 1.

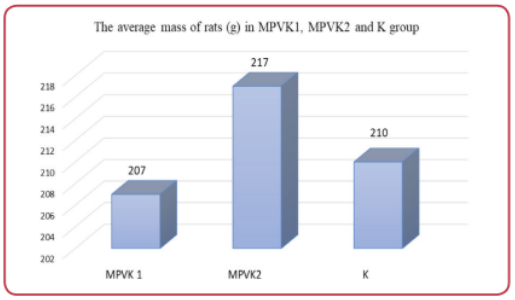


Figure 1: The average weight of Wistar rats in the MPVK 1, MPVK 2 and K group

Treatment group 1 (MPVK1) rats were given the yellow kepok banana peel extract at a dose of 80 mg / 200 g BW with 0.3 % NaCMC solvent and treatment group 2 (MPVK2) rats were given 160 mg / 200 g BW yellow kepok banana peel extract with 0.3 % NaCMC solvent. The control group (K) was given 0.3 % NaCMC.

The minimum level of MDA in the MPVK 1 group was 1.01, while the maximum level was 7.67 and its average was 2.60, respectively. The minimum level of MDA in the MPVK2 group was 1.5, while its maximum level was 2.10 and the average was 1.81. The minimum level of MDA in the control group (K) was 2.19, the maximum level was 3.93 and the average was 3.27. The lowest mean level of MDA was in the MPVK2 group, as shown in Figure 2.

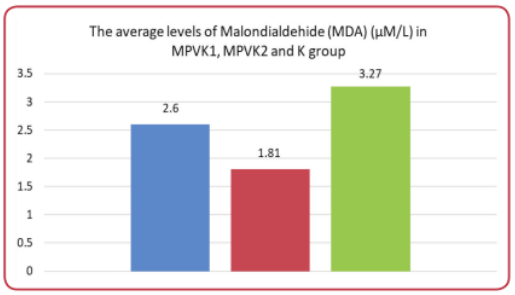


Figure 2: The average levels of malondialdehyde (MDA) of rats in MPVK1, MPVK2 and K group

Treatment group 1 (MPVK1) rats were given the yellow kepok banana peel extract at a dose of 80 mg / 200 g BW with 0.3 % NaCMC solvent and treatment group 2 (MPVK2) rats were given 160 mg / 200 g BW yellow kepok banana peel extract with 0.3 % NaCMC solvent. The control group (K) was given 0.3 % NaCMC.

The average levels of nitric oxide (NO)

The minimum level of NO in the MPVK1 group

was 88, meanwhile the maximum level was 131.28 and the average was 112.73. The minimum level of NO in the MPVK2 group was 221.28, while the maximum level was 353.21 and the average was 286.17. The minimum level of NO in the K group was 101.95, meanwhile, the maximum level was 120.28 and the average was 111.74. The lowest average NO level was in group K, as shown in Figure 3.

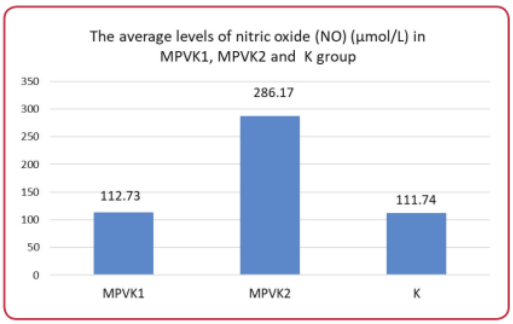


Figure 3: The average levels of nitric oxide (NO) in MPVK 1, MPVK 2 and K group

Treatment group 1 (MPVK1) rats were given the yellow kepok banana peel extract at a dose of 80 mg / 200 g BW with 0.3 % NaCMC solvent and treatment group 2 (MPVK2) rats were given 160 mg / 200 g BW yellow kepok banana peel extract with 0.3 % NaCMC solvent. The control group (K) was given 0.3 % NaCMC.

The average number of ulcers

The minimum number of ulcers in the MPVK1 group was 0, with its maximum was 3 and the average was 0.45. The number of ulcers in the MPVK2 group was 0, a maximum of 1 and its average was 0.04. The number of ulcers in the control group (K) was 0, a maximum of 7 and its average was 3.55. The lowest mean of number ulcers was in the MPVK 2 group, as seen in Figure 4.

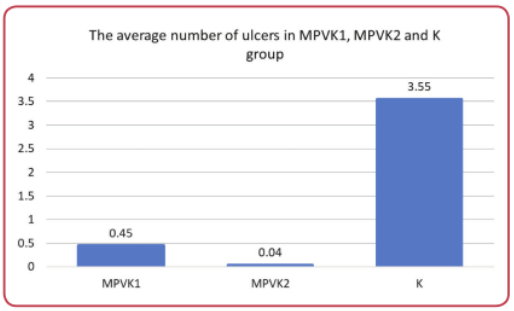


Figure 4: The average number of ulcers in the MPVK1, MPVK2 and K group

Treatment group 1 (MPVK1) rats were given the yellow kepok banana peel extract at a dose of 80 mg / 200 g BW with 0.3 % NaCMC solvent and treatment group 2 (MPVK2) rats were given 160 mg / 200 g BW yellow kepok banana peel extract with 0.3 % NaCMC solvent. The control group (K) was given 0.3 % NaCMC.

A macroscopic picture of gastric mucosal deterioration is shown in Figure 5. The results showed that the highest average number of ulcers was found in the control group.

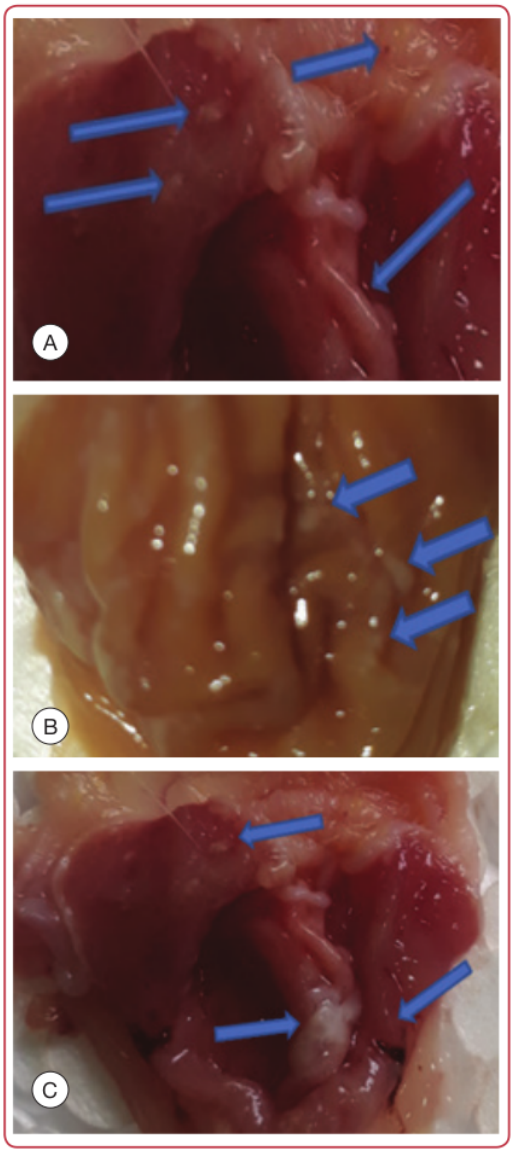
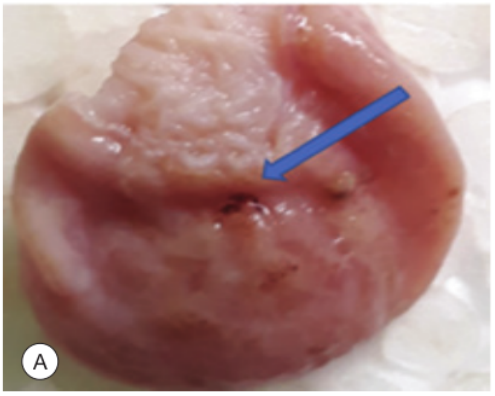


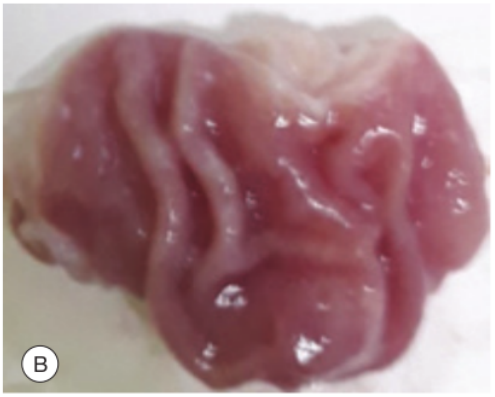
Figure 5 (A, B, C): Gastric macroscopic characteristics observed in the control group. The signs of hypoxaemia, bleeding and gastric mucosal ulcers were displayed by the blue arrows

The control group rats were given 0.3 % NaCMC solvent.

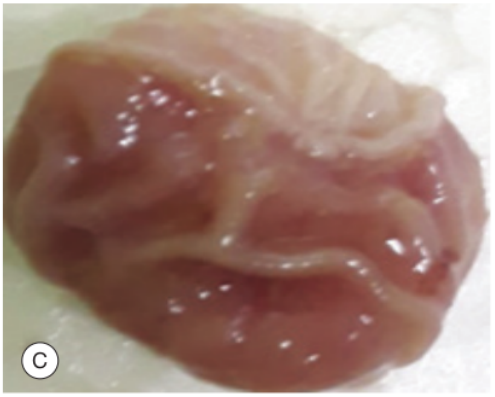
The results of gastric macroscopic examination in treatment of group 1 and 2 are shown in Figure 6.



Minimal bleeding for MPVK1 group



Absence of bleeding and ulcers in MPVK2 group



Absence of bleeding and ulcers in MPVK2 group

Figure 6: *Gastric macroscopic characteristics observed in the treatment groups*

A. Macroscopic characteristics of stomach in the MPVK1 group showing minimal bleeding (pointed by the blue arrow), with fewer ulcers than that in the control group; B and C, showing macroscopic characteristics of the stomach in the MPVK2 group which did not perform bleeding and ulcers. Treatment group 1 (MPVK1) rats were given the yellow kepok banana peel extract at a dose of 80 mg / 200 g BW with 0.3 % NaCMC solvent and treatment group 2 (MPVK2) rats were given the yellow kepok banana peel extract at a dose of 160 mg / 200 g BW with 0.3 % NaCMC solvent.

Table 1 shows the results of the One-Way ANOVA test. It revealed that there were significant distinctions in the average level of MDA and NO and the number of ulcers in the 3 group of experimental rats; MPVK 1, MPVK 2 and K.

Table 1: One-Way ANOVA test results has displayed the average values of MDA and NO, accompanied by the number of ulcers in the experimental groups MPVK1 and MPVK 2 and control group K

Parameter	MPVK1	MPVK2	K	p-value
MDA	2.60	1.81	3.27	0.013
NO	112.73	286.17	111.74	< 0.001
Number of ulcers	0.45	0.04	3.55	< 0.001

MDA: malondialdehyde; NO: nitric oxide; Treatment group 1 (MPVK1) rats were given the yellow kepok banana peel extract at a dose of 80 mg / 200 g BW with 0.3 % NaCMC solvent and treatment group 2 (MPVK2) rats were given 160 mg / 200 g BW yellow kepok banana peel extract with 0.3 % NaCMC solvent. The control group (K) was given 0.3 % NaCMC.

Treatment group 1 (MPVK1) rats were given the yellow kepok banana peel extract at a dose of 80 mg / 200 g BW with 0.3 % NaCMC solvent and treatment group 2 (MPVK2) rats were given 160 mg / 200 g BW yellow kepok banana peel extract with 0.3 % NaCMC solvent. The control group (K) was given 0.3 % NaCMC.

Discussion

An induction of free radicals and oxidative stress causes gastric mucosal deterioration.[2] Further, an imbalance of aggressive and defensive factors can cause gastric mucosal ulcers. The aggressive factor is more dominant than the defensive factor. The existence of free radicals is part of the aggressive factor.[1] An example of free radicals is NSAIDs. Acetylsalicylic acid works by blocking certain natural substances in the body to reduce pain and swelling. Acetylsalicylic acid is an irritant.[4-6] Acetylsalicylic acid causes a defect in the mucosal barrier and back diffusion of H^+ ions occurs. Histamine is stimulated to secrete more gastric acid, resulting in dilation and increased permeability of capillaries, gastric mucosal damage, acute or chronic gastritis and gastric mucosal ulcers.[16]

The experimental animal model of gastric mucosal ulcers in this study was carried out by inducing acetylsalicylic acid. 5% acetylsalicylic acid was given at a dose of 1500 mg/kg BW in all study groups. The results showed the highest average number of ulcers was found in the control group. The minimum number of gastric ulcers in the control group (K) was 0, the maximum number of ulcers was 7 and the average ulcer was 3.55.

The detrimental effect of free radicals that cause biological damage is oxidative stress. Cells exposed to oxidative stress will activate defence mechanisms to survive.[7, 17] Lipid peroxidation itself is the result of the performance of free radicals and this parameter is the most accessible to measure.[2, 11] Lipid peroxidase can damage membrane structures, it leads changes in permeability, inhibits metabolic processes and transform ion transport as well.[11] The measurement of lipid peroxidation level is carried out by measuring the final product, one of which is MDA. The accumulation of MDA is an early indicator of the mechanism of cell and tissue deterioration. MDA as final product of the lipid peroxidation process is used as an indicator of cell deterioration in the stomach due to the oxidative stress.[9-11]

The results of this study have revealed that the minimum level of MDA in the control group (K) of 2.19 is the highest among those three groups, as well as the maximum and the average level of MDA.

Kepok and Uli banana peel extracts had increased superoxide dismutase (SOD) activity and decreased MDA levels in hypercholesterolemic rats.[18] Kepok banana peel extracts decreased MDA levels in male mice (*mus musculus*) that was exposed to cigarette smoke.[14] Kepok banana peel contains flavonoid and phenolic antioxidants, the antioxidant content of flavonoids and phenolics showed hepatoprotection in acetylsalicylic acid-induced rats.[19] Other antioxidants component in kepok banana peels are flavonoids, tannins, saponins and polyphenols. Tannins are useful in minimising gastric acid secretion and have cytoprotective effect.[5] Other benefits of tannins are the increasing tissue reconstruction, gastric acid production inhibition and the inhibition of *Helicobacter pylori* activity. Further, saponins also inhibit gastric acid production and minimise gastric fluid pH levels.[8, 16, 20]

Kepok banana peel extract reduces oxidative stress in peptic ulcers through the antioxidant pathway.[19] Prevention and treatment of gastric mucosal ulcers by exploring natural products is something that is very impressive. The rats that were given kepok banana peel extract performed the increase of protection phenomena against acetylsalicylic acid induction by elevating the antioxidant level of NO. Acetylsalicylic acid converts hydroperoxyl to hydroxy fatty acids which is culminated from lipid peroxidation of cell destruction. The release of damaging free radicals likely occurs resulting in the death of tissue cells in the stomach. The ulceration effect of superficial epithelial cells on the gastric mucosa constructs the base of gastric ulceration.[14] The kepok banana peel extract exhibits its gastroprotection by increasing mucosal defence factors by increasing the body's antioxidant levels, NO.[14, 16]

This study revealed that the rats given kepok banana peel extract had increased average level of NO antioxidants compared to the control group, namely MPVK1 (112.73) and MPVK2 (286.17). The lowest average NO level was found in the control group, that is 111.74. There is a significant distinction in the mean of NO in the three groups with $p < 0.001$. The average number of ulcers in the treatment group was lower than in the control group. Hence, the mean number of ulcers in the MPVK1 group was 0.45. The average number of ulcers in the MPVK2 group was 0.04. The mean number of ulcers in control group (K) was 3.55. Meanwhile, the low mean number of ulcers was found in MPVK2 group. There was a significant distinction in the mean number of ulcers in the three groups with $p < 0.001$.

Conclusion

There is a significant distinction in the mean value of MDA, NO as well as the distinction of number of ulcers among the three groups. Therefore, kepok banana peel extract has good efficacy in reducing the markers of gastric damage in Wistar rats.

Acknowledgements

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Conflict of interest

None.

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