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Research Article

Protective Effect of Combination Black Seed Oil (*Nigella sativa)* and Honey on the Duodenum of Rats Exposed to Cisplatin

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ABSTRACT

Background: Cisplatin (CP) is an anticancer agent with various side effects, including duodenal mucosal damage. It is well known that black seed oil (BSO) and honey (H) are rich in antioxidants and have a mucosal protective effect. **Purposes:** To determine the protective effect of the BSO and H combination on the histopathology of duodenal rats given CP exposure. **Methods:** Randomized post-test-only control group design was used in this study. A total of 30 rats were divided into ten groups, namely the control group (K), treatment groups (P1-P8), and cisplatin group (C). The treatment groups were given BSO and H orally for 21 days, and CP was given intraperitoneally on day 18 to the treatment and cisplatin groups. On day 22, the duodenal tissue was taken for preparation and histopathological assessment. Data analysis using IBM SPSS v.24 and Compusyn program. **Result:** There were significant differences in the duodenal mucosa damage scores of the P1-P8 group compared to the C group ($p<0.05$). The combination index of P7 exerted a synergism effect (CI < 1). **Conclusion:** The combination of BSO and H exerted a protective effect on the histopathological of rats' duodenal tissue induced with CP, and the combination of BSO 2 mL/KgBW and H 3.7 mL/KgBW exerted a synergism effect.

Keywords: anticancer, antioxidant, drug interaction, medicinal plant

INTRODUCTION

Cisplatin is an antineoplastic agent with highly toxic, but it is one of the most widely used chemotherapeutic agents for hematology and malignancies of solid tumor (1). Cisplatin toxicity has been described as a function of DNA binding, followed by single-stranded DNA breaking (2). According to Gao J et al., cisplatin causes severe mucosal erosion and ulcers, accompanied by epithelial loss and bleeding. It has been found that the mechanism of mucosal injury is caused by many factors, including inflammation, mucosal oxidative stress, and apoptosis of epithelial cells (3).

The duodenum is an organ that has many mitochondria. Mitochondria play an important role in the induction of cell death. Mitochondria-rich mucosal cells undergo a high degree of apoptosis upon administration of cisplatin. Apoptosis is cell death by activating enzymes that damage the cell's nuclear DNA. Cell fragments that undergo apoptosis will be released, resulting in damage in desquamation, erosion, and ulceration. This is supported by

research conducted by Qian et al. showing that mitochondrial density and cell sensitivity correlate with cisplatin toxicity. Cell death was more prevalent in areas with richer mitochondria cells, such as the duodenum (4).

Black Seed Oil (BSO) is used in ancient medicine for several ailments (5). Bioactive compounds on it, such as thymoquinone and polyunsaturated fatty acids, are known to have various positive effects, such as antioxidants, cell repair, and healing of various diseases (6). Honey is a natural product formed from the nectar of flowers by honeybees. Evidence indicates that honey can exert several health-beneficial effects, including antioxidant, anti-inflammatory, respiratory, GI tract, cardiovascular, and nervous system protective effects (7). According to Siddiq AM, cisplatin produces oxidative stress, characterized by an increase in malondialdehyde levels. Still, after the administration of single or combination doses of black seed oil and honey, the oxidative stress levels decreased (1).

Previous studies showed that the administration of BSO or H in single or combination doses would provide a protective effect and repair the gastrointestinal mucosa damage caused by cisplatin (8,9). Our study aim is to assess the protective effect of the combination of BSO and H on duodenum histopathology tissue of rats induced by cisplatin.

METHODS

This experimental study was conducted in the microscopic laboratory, Faculty of Medicine, Tanjungpura University, Pontianak. Randomized post-test only control group design was used in this study by dividing 30 Wistar strain male rats (*Rattus norvergicus*) into ten groups of treatment, as follows: normal control group (K), BSO 1 mL/KgBW (P1), BSO 2 mL/KgBW (P2), H 3,7 mL/KgBW (P3), H 7,4 mL/KgBW (P4), BSO 1 mL/KgBW and H 3,7 mL/KgBW (P5), BSO 1 mL/KgBW and H 7,4 mL/KgBW (P6), BSO 2 mL/KgBW and H 3,7 mL/KgBW (P7), BSO 2 mL/KgBW and H 7,4 mL/KgBW (P8), and Negative control groups (C). P1-P8 groups were given BSO and H orally for 21 days. On day 18, cisplatin 8 mg/KgBW was given intraperitoneally to negative and P1-P8 groups, while the normal control group was given NaCl 0,9% 1 mL/KgBW intraperitoneally. P1-P8 groups were given BSO and H orally for 21 days. On day 18, cisplatin 8 mg/KgBW was given intraperitoneally to negative and P1-P8 groups, while the normal control group was given NaCl 0,9% 1 mL/KgBW intraperitoneally.

Histopathological preparations of the duodenum were made using Hematoxylin-Eosin staining. Duodenal histopathological observations on changes in epithelial structure were observed microscopically with 40x magnification and using Barthel Manja's mucosal integrity score (10). Data were analyzed statistically using SPSS ver. 23 for windows and conducted by Kruskal Wallis followed by Mann-Whitney test, a probability level of $p<0.05$ was selected as indicator of statistical significance. Analysis of the combination index using Compusyn 2005 for Windows. This research was carried out after being reviewed and approved by the Division of Ethical Studies, Faculty of Medicine, Tanjungpura University. The certificate number for passing the ethical review used is 7197/UN22.9/DL/2018.

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RESULTS

In this study, observations were made to the epithelial cells of the duodenal mucosal which underwent histopathological changes using a light microscope at 40x magnification. The histopathological of duodenum in normal control group showed no damage. In contrast, the result from the treatment group given cisplatin expressed damages such as desquamation, erosion, and ulceration, (Figure 1A-D). The amount of damage to the duodenal mucosa in each visual fields was totaled and averaged for each rat. Then the average of each rat is added up and the group average is made. The average of damage for each group can be seen in Figure 2.

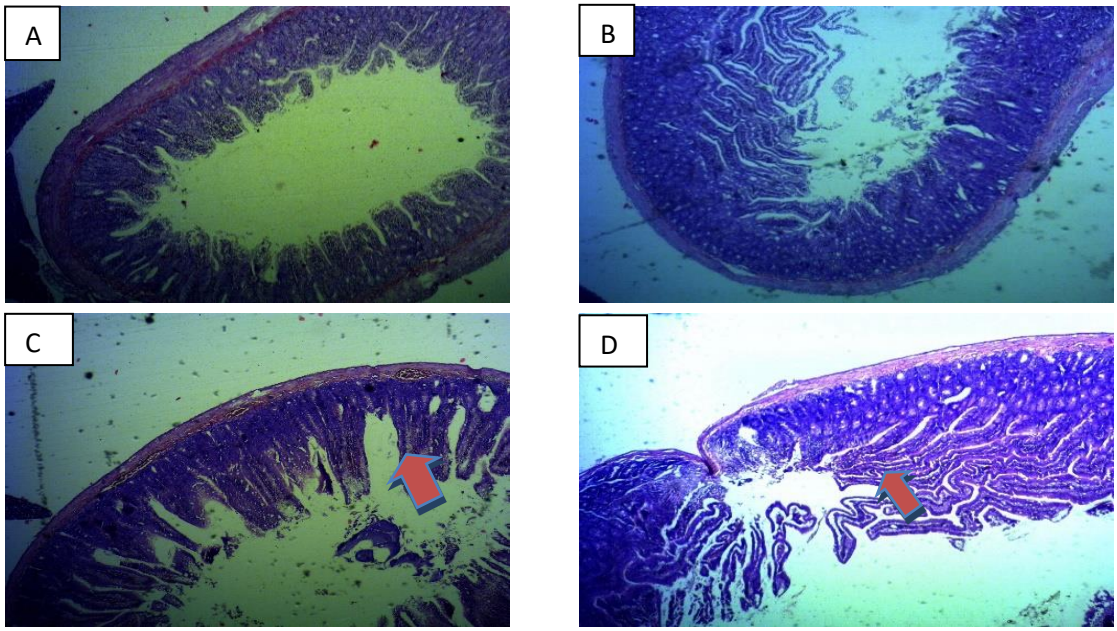

Figure 1. Duodenal Histopathological observations. (A) Normal epitel. (B) Desquamation or the release of epithelial elements (C) Erosion or the loss of the intestinal epithelium at a certain focus without loss of the muscularis mucosa (D) Ulceration, i.e. breaking of the surface of the mucosa epithelium

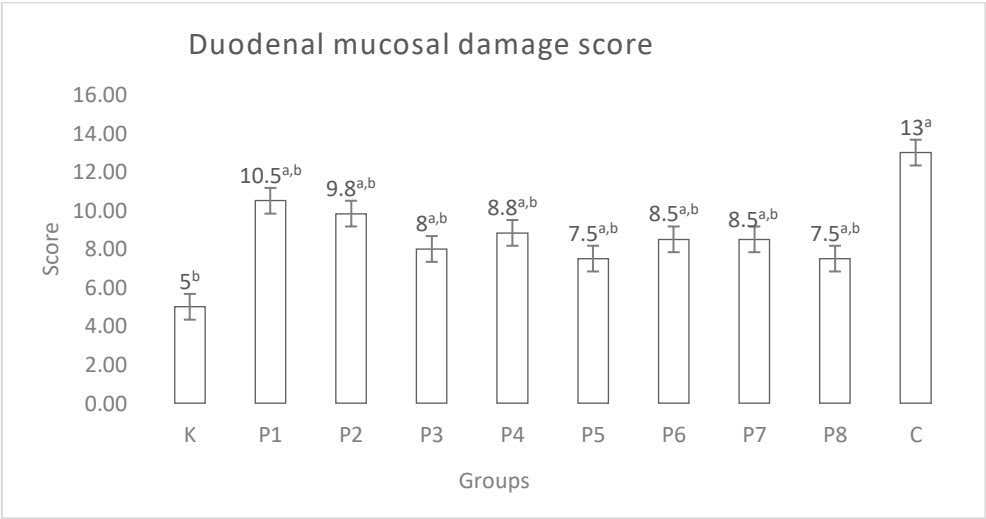

Figure 2. Duodenal mucosal damage score in all groups. Kruskal Wallis ($p \leq 0.05$). a = significantly from group K ($p < 0.05$); b = significantly from group C ($p < 0.05$)

The results showed that all treatment groups (P1-P8) had a milder degree of mucosal damage than group C ($p<0.050$). Examination of the duodenal mucosa in the combination group (P5-P8) showed less damage to the duodenal mucosa than the single dose group (P1-P4). Results from the CI analysis in the P7 combination group showed a synergistic effect, whereas in P5, P6, and P8 combination groups indicated an antagonism effect. CI results can be seen in table 1.

Table 1. Combination Index (CI).

CI		H (mL/KgBW)	
		3.7	7.4
BSO (mL/KgBW)	1	1.6	1.4
	2	0.9	3.2

CI (Combination index, BSO (black seed oil), H (Honey).

DISCUSSION

Based on histopathological observations of the duodenal showed that intraperitoneally of cisplatin 8 mg/KgBW caused duodenal mucosa damage characterized by the form of desquamation, erosion, and ulceration. Damage to the duodenal mucosa can result from changes in the mucus, which protects the duodenal mucosa. The accumulation of mucus is critical in protecting the GI epithelium against various irritants. Results from the previous study indicated that the administration of cisplatin led to a decrease in mucin content in all parts of the GI tract (11).

Cisplatin works by binding to guanine in DNA, forming cross-links, and causing cytotoxic lesions that inhibit polymerase for DNA replication and RNA synthesis. Cisplatin can also reduce levels of non-enzymatic antioxidants, increase lipid and protein oxidation and interfere with mitochondrial complex enzyme activity, which can increase reactive oxygen species (ROS) and decrease ATP, resulting in oxidative stress (12). If there is an increase in oxidative stress and impaired formation of ATP, the impact of its derivatives will cause various abnormalities in cell function. In addition, oxidative stress can cause membrane damage due to lipid peroxidation and disruption of cell biochemical processes due to cross-linking of free radicals (13).

BSO and H administration alone or in combination for 21 days protected the duodenum against cisplatin exposure and showed a statistically significant difference to group C. Bioactive compounds on BSO, like thymoquinone and polyunsaturated fatty acids, while in H, like polyphenols and tocopherols, have various positive effects such as antioxidants, cell repair, and healing of various diseases (6). In Mirela B's research, the analysis of the active component content between a mixture of BSO and H had a higher concentration of total phenols than pure honey with a ratio of 3:2 and a significant amount of Thymoquinone (14).

The results in P1-P2 groups (BSO 1 and 2 mL/Kg) were milder than the C group. This result was in line with research by Egilmez et al., inflammatory cell infiltration, vascular dilatation, superficial erosion, and exudates was better in rats given cisplatin plus BSO than those given only cisplatin. BSO's possible mechanism of action in preventing damage to the

duodenal mucosa caused by cisplatin-induced was anti-inflammatory, antioxidant, and cytoprotective (15).

A single dose of honey also provides a protective effect on the rat's duodenal tissue. These results align with research conducted by Ibrahim et al., which revealed that giving honey could prevent kidney damage due to cisplatin administration (16). Phenolic acid compounds and flavonoids mainly play the antioxidant activity of honey. Flavonoid compounds are included in the polyphenolic compounds, which are polar and can dissolve in water. Flavonoids and phenolic acids have antioxidant activity through free radical scavenging. Based on their structure, flavonoids have more than one phenol group. They have conjugated double bonds, so they can ward off free radicals, which decrease lipid peroxidation and increase the activity of antioxidant enzymes such as SOD, CAT and GPx (17,18).

Duodenal histopathological examination results in the combination group (P5-P8) showed a better protective effect than the single-dose group. CI values in the P7 group (BSO 2 mL/Kg and H 3.7 mL/Kg) demonstrated a synergistic effect (CI < 1). This study showed results that were in line with research conducted by Alkadri and Chikrista, that the combination of BSO and H was able to provide a protective effect against histopathological damage to liver and kidney tissue due to exposure to cisplatin, and the CI value also showed a synergistic effect. Based on Alkadri, it was found that there was a lot of hepatocyte damage that experienced hydropic degeneration and necrosis (9). Chikrista's study found that there was a lot of damage to the proximal kidney tubules, such as hydropic degeneration, necrosis, cast formation, and loss of brush border after exposure to cisplatin (21).

Our study revealed that the administration of the combination of BSO and honey in variety provides a better effect than a single treatment of BSO or honey. This result is in line with a study conducted by El-Kholy et al., a better protective effect on the liver was obtained after the combination of BSO and honey was given (20). This study was an experimental study, which was carried out by comparing histopathological preparations between the normal control group, negative control group, and the exposure group so that the types of damage that occurred could be compared. In this study, there are research limitations, that is less variation in doses. More dose variations are expected to determine the medicinal and combination properties.

CONCLUSION

A combination of BSO and honey has a better protective effect on the duodenal tissue of rats than a single treatment exposed to cisplatin. The combined BSO 1 mL/KgBB and honey 3.7 mL/KgBB showed a synergistic effect. Further research with a more varied range of BSO and honey doses to determine medicinal and combination properties, and the need to examine the single content of BSO and honey quantitatively to see the protective effect on the duodenum.

CONFLICT OF INTEREST

The authors do not have a conflict of interest and that do not have affiliations or relationships with any organization or entity that could raise biased questions or statements in the discussion and conclusion sections of the paper.

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