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Effect of *Nigella sativa* Extracts in Liver Cell of *Rattus norvegicus* Induced by Piroxicam

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ABSTRACT

Piroxicam can cause side effects, especially in the liver, so that it can cause cell damage and impair the function and work of the liver organs. One way to minimize liver cell damage is by giving Nigella sativa. This study aims to analyze the effect of Nigella sativa extract on the level of liver cell damage in rats (Rattus norvegicus) induced by Piroxicam. This study used 80 male rats and was divided into three groups, namely negative control (K-), Piroxicam non-extract (P-), and Piroxicam plus extract (P +). Nigella sativa extract is given orally at a dose of 2 g/kg BW; 3 g/kg BW; 4 g/kg BW; 5 g/kg BW, and 6 g/kg BW, while the Piroxicam dosage given is 1 g/kg BW and 3 g/kg BW. The liver cell damage was done by observing the microscopic image. The results showed that the more doses of Piroxicam were given, the higher the level of cell damage. Along with increasing the amount of black cumin extract, the cell damage is reduced.

Introduction

Piroxicam is a crystalline powder colorless, odorless, bitter, and in yellow monohydrate form [1]. Piroxicam is one of the Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) is an oxicam derivative as well as a nonselective inhibitor cyclooxygenase (COX) [2] and work by inhibiting enzymes cyclooxygenase 1 and 2, resulting in production prostaglandins (PGE2) and prostacyclin (PGI2) ones is an inflammatory mediator that results in the occurrence of vasoconstriction of blood vessels can decrease [3]. Drug interactions can change drug activity, either by increasing toxic effects or decreasing therapeutic effects [4]. The treatment of Piroxicam can also reduce mucus secretion in the stomach. Due to the nature of the acid, it is corrosive to damage the gastric mucosal epithelium [5]. It has the potential to cause side effects on the liver [6]. The longer the half-life of Piroxicam, the easier it will be for accumulation (buildup) in a person's body which will cause toxic effects [7]. If the harmful effects are enormous, the liver as a detoxification organ cannot perform its function optimally.

One way to minimize liver cell damage is by giving *Nigella sativa*. *Nigella sativa* contains significant components, including thymoquinones, fatty substances, protein, potassium, melantin (saponins), nigellin (bitter substances), nigelon, and tanning substances [8]. Timoquinone has antibacterial, antioxidant, antihistamine, antiinflammatory, antidiabetic, analgesic, antipyretic and antineoplastic effects. *Nigella sativa* contains linoleic acid or omega 6 in fixed oil as other active substances besides timoquinone [9]. *Nigella sativa* therapy from Habasyah 2000 mg/day for 50 days can significantly reduce systolic and diastolic blood pressure before and after treatment [10]. *Nigella sativa* oil is very influential in the process of reducing the scale of pain, exudate, and malodor in cancer wounds [11].

Nigella sativa has antiviral, antifungal, antibacterial, antihypertensive, and antiparasitic properties. *Nigella sativa* extract has also been shown to increase the non-specific and specific immune system [12]. *Nigella sativa* contains an active ingredient in nigelon, which functions as a stabilizer in the immune system during growth and suppresses antihistamines that cause asthma, bronchitis, neurodermatitis, and allergies [13].

Nigella sativa seed extract with thymoquinone as its primary active substance has a cytotoxic effect on human cancer cell lines, liver anti-cancer, immunomodulation (increased cell function (T and B lymphocytes, NK cells, macrophage cells, CTL cells, IL-2 and three productions, TNF-β4) and induces apoptosis. Thymoquinone is also known to inhibit cancer cells that have been resistant to previous anti-cancer treatments such as cisplatin and doxorubicin. Other research also states that *Nigella sativa* extract contains fatty acids which in vitro have a cytotoxic effect of 50% against liver cancers of *Ehrilch Ascites Carcinoma* (EAC), *Dalton's Lymphoma Ascites* (DLA), and malignant cancer cells Sarcoma-180 and simultaneously in vivo can completely inhibit EAC development [14].

Nigella sativa has antioxidant potential by having radical scavenging abilities, effective in nonenzymatic lipid peroxidation, and deoxyribose degradation. Another study explains that giving *Nigella sativa* extract to a sample of mice as much as 1-2 g/kg BW / day for ten days can show a therapeutic effect [15].

Experimental Method

This study used 80 male rats with an average body weight of 180-200 grams. The rats were grouped into three groups, namely K- (without Piroxicam and extract), P- (Piroxicam without extract), and P+ (given Piroxicam then extract). The doses of Piroxicam used were 1 g/kg BW and 3 g/kg BW, while the *Nigella sativa* extract was given in five dose variations, namely 2 g/kg BW; 3 g/kg BW; 4 g/kg BW; 5 g/kg BW and 6 g/kg BW. Each group consists of five rats.

Piroxicam Treatment. Piroxicam given to rats is a finished powder that has been packaged and sold in the market, taking into account the composition contained in it. One Piroxicam capsule contains 10 mg. The dose of Piroxicam given to rats was calculated based on the bodyweight of each rat. Piroxicam was given once a day for seven days before the rats were given black cumin extract by force-feeding the rats using a gastric swab.

***Nigella sativa* Extract Treatment.** *Nigella sativa* extract given to rats is a finished powder that has been packaged and sold in the market, taking into account the composition contained in it. One *Nigella sativa* extract capsule contains 600 mg. The dose of *Nigella sativa* extract given to

rats was calculated based on the bodyweight of each rat. The *Nigella sativa* extract was shown once a day for seven days after the rats were given Piroxicam by force-feeding the rats using a stomach swab.

Preparation of Histology. The rats that had been operated on had their hepatic organs removed. The rat's liver was dehydrated and then cut with a microtome. Pieces of the liver were rehydrated, and HE stained. Furthermore, it can be observed under a microscope. The method to calculate the cell damage is to look at five fields of view on each preparation. Each area of view is characterized by the number of normal cells and cell damage such as parenchymal degeneration or rupture of hepatocyte cell walls, binuclear cells, or the joining of two hepatocyte cells. The boundary between the cell walls is not known.

Result and Discussion

The results of observing the histological image of rat liver cells showed that the treatment of Piroxicam doses could cause liver cell damage in rats.

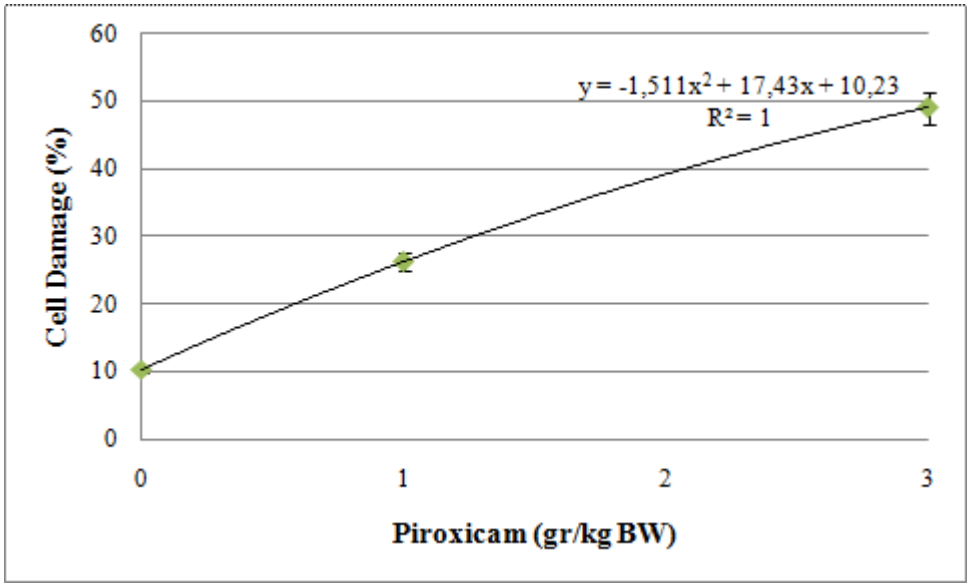


Figure 1. The Relationship between The Number of Doses of Piroxicam on Rat Liver Cell Damage

In control animals, the cell damage reached 10.23%. It is because the conditions of the rats are different from each other in the acclimation process. The acclimatization process allows rats to adapt to survive in different environmental conditions from their place of origin. The adaptation process emphasizes the phenotypic changes of rats. It can be seen in Figure 1 that the more doses of Piroxicam are given, the greater the percentage of cell damage. At the amount of Piroxicam 1 g/kg BW, the cell damage reached 26.16%. An increase in the rate of cell damage also occurred when the rats were given a dose of Piroxicam 3 g/kg BW, which was 48.95%.

Figure 2 shows a microscopic view of rats, liver cells taken at 100x magnification. In control rats (standard), shown in Figure 2a, the central vein is oval, but there is a blood clot. The arrangement of hepatocytes and sinusoid cells is mainly regular. However, more and more doses of Piroxicam are given, causing the centralis and sinusoid veins to become dilated, even

more irregular in shape. Piroxicam dosage also has an impact on the occurrence of blood clots in the central vein. It is evidenced by the small amount of blood taken from the heart of the rats. Hepatocyte cells also experience damage (necrosis), such as parenchymal degeneration or rupture of hepatocyte cell walls, binuclear cells, or the joining of two hepatocyte cells. The boundary between the cell walls is not known. The number of normal cells will decrease with the addition of the Piroxicam dose, or the number of damaged cells will increase.

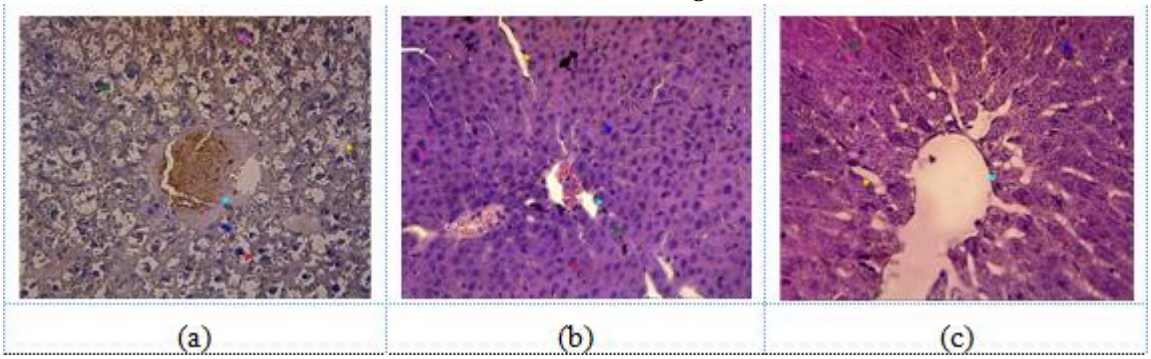


Figure 2. Microscopic Picture of Rat Liver Cells (a) Control (b) Piroxicam 1 g/kgBW (c) Piroxicam 3 g/kgBW

Figure 3 shows the percentage of rat liver cell damage, where there was a decrease in cell damage from 26.16% to 15.71% in Piroxicam 1 g/kg BW treatment and 48.95% to 28.45% in Piroxicam 3 g/kg BW.

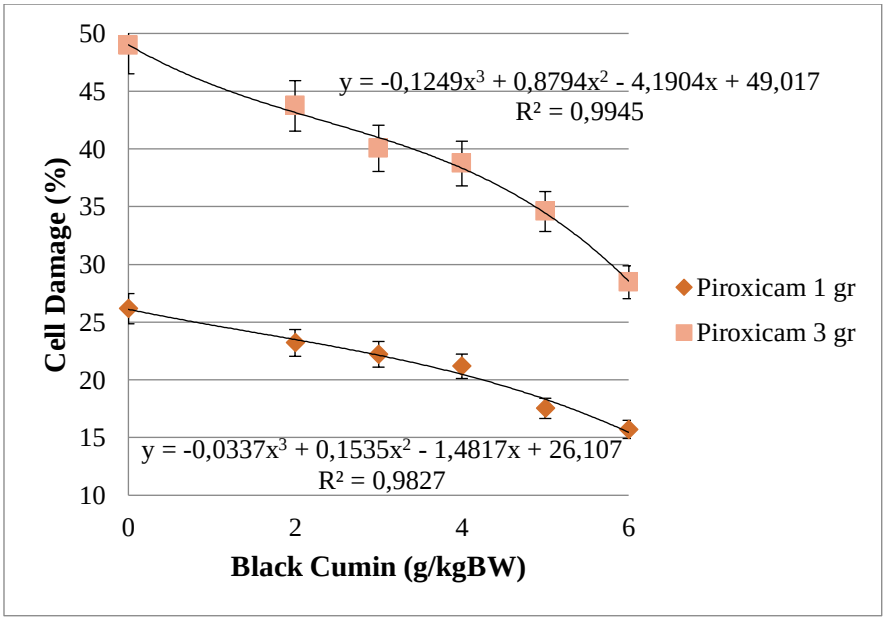
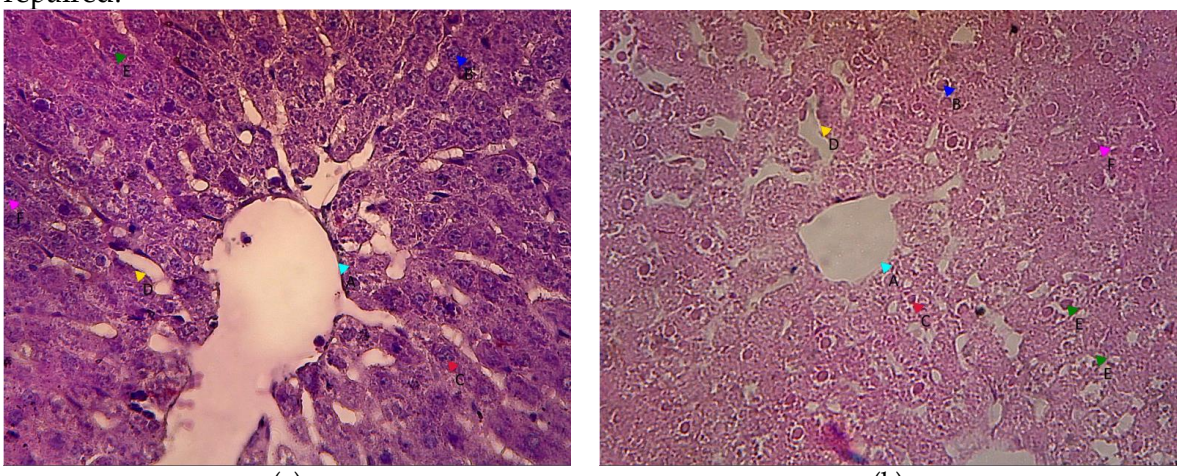


Figure 3. The Relationship between The Number of Doses of Black Cumin Extract on Rat Liver Cell Damage

These results were also confirmed by the microscopic image of rat liver cells, shown in Figure 4. Giving *Nigella sativa* extract can repair cell damage, such as in the shape of the central veins and sinusoids, which are close to the typical form and do not occur blood clots.

The number of damaged cells will be reduced, even though these cells are not completely repaired.



(a) (b)

Figure 4. Microscopic Picture of Rat Liver Cells (a) Piroxicam (b) Piroxicam + *Nigella sativa*

Lipid peroxidation causes hepatic cell necrosis. In pycnosis, damaged hepatocytes are slightly black and do not yet have a cell membrane. Some cells that undergo picnosis are more significant than normal hepatocyte cells, and some are smaller than normal hepatocyte cells. Hepatocyte cells that experience necrosis shrinks in shape so that they become irregular. The necrosis causes the nucleus to swell, and the cytoplasm breaks so that the cell's contents, such as the SGPT enzyme, enter the extracellular tissue due to interference with the sodium pump due to a lack of ATP. ATP plays an essential role in the integrity of hepatocyte cells. If the ATP level is low, the intracellular enzymes will be released from the blood, causing damage to the liver [16].

Nigella sativa inhibits the breakdown of liver cells due to the presence of free radicals. *Nigella sativa* also can reduce the level of damage to rat liver cells. Several chemical compounds in *Nigella sativa* act as antioxidants that can ward off free radicals [17].

Conclusion

Piroxicam has side effects, especially on the liver. The more doses of Piroxicam were given, the higher the level of cell damage. Along with increasing the dose of *Nigella sativa* extract, the cell damage is reduced.

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