

## HEPATO-NEPHROPROTECTIVE ACTIVITY OF *NIGELLA SATIVA* OIL ON PARACETAMOL-INDUCED NEW ZEALAND RABBITS (*ORYCTOLAGUS CUNICULUS*)

NADHIRA M. DINAR<sup>1</sup>, SHEILA PRATIWI<sup>1</sup>, RAIN KIHARA<sup>1</sup>, NADIA G. PARAMITA<sup>1</sup>, NAZILLA R. FATHURRAHMAN<sup>1</sup>, JUTTI LEVITA<sup>1,2\*</sup>

<sup>1</sup>Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang km.21 Jatinangor, West Java, Indonesia 45363, <sup>2</sup>Scientific Consortium for Drug Discovery and Development, Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung Sumedang km.21 Jatinangor, West Java, Indonesia 45363  
Email: jutti.levita@unpad.ac.id

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### ABSTRACT

**Objective:** The objective of this work was to investigate the hepato-nephroprotective activity of *Nigella sativa* (Ranunculaceae) oil on paracetamol-induced New Zealand rabbits (*Oryctolagus cuniculus*).

**Methods:** Hepato-nephroprotective activity of *Nigella sativa* oil was demonstrated on six groups of paracetamol-induced New Zealand rabbits (*Oryctolagus cuniculus*) aged 3-4 mo, three in each group (2 males, 1 female). Group I was normal control (water 1.0 ml/kg of body weight per oral), group II was negative control (water 1.0 ml/kg of body weight per oral), group III was positive control (silymarin 100 mg/kg of body weight per oral), group IV-VI were treated with *Nigella sativa* oil (NSO) dose of 0.5 mg/kg of body weight, 1.0 mg/kg of body weight, and 2.0 mg/kg of body weight per oral, respectively, for 15 d. At the 16<sup>th</sup> day, rabbits in group II-VI were induced with paracetamol at a dose of 600 mg/kg of body weight per oral. At the 23<sup>rd</sup> day the animals were measured for their clinical biochemistry parameters and histological examination.

**Results:** Paracetamol administration dose of 600 mg/kg of BW resulted in the elevation of serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and ureum-N levels of the animals, particularly in group II which was treated only with paracetamol. Normal histology of the liver defines the clear shape of the terminal hepatic venule (THV)/central vein (CV) and sinusoids, whereas that of the kidney defines clear shape of the Bowman capsule and glomerulus shape. Qualitative histological examination of the liver showed that the THV/CV in all groups was normal, however in the paracetamol-treated group, the sinusoids were dilated, necrosis and mass apoptosis were detected. Dilated sinusoids were observed in the silymarin group and in the lower and medium doses of NSO groups. In the highest dose of NSO group the THV/CV and sinusoids were normal, but a local apoptosis and fat degeneration were detected. Qualitative histological examination of the kidney indicated that there was no abnormality of the glomerulus shape, however, mass apoptosis and local necrosis of the kidney were found in the paracetamol-treated group and the silymarin-treated group. The lowest dose of the NSO-treated group showed a normal shape of glomerulus and Bowman capsule, normal apoptosis. No necrosis was observed in the rabbit's kidney. Higher doses of NSO groups indicated a normal glomerulus shape and Bowman capsule, mass apoptosis and local necrosis.

**Conclusion:** In this study, *Nigella sativa* oil could maintain the normality of the THV/CV and sinusoids in the liver of paracetamol-induced New Zealand rabbits (*Oryctolagus cuniculus*). Normal glomerulus shape and Bowman capsule were also confirmed in the kidney of paracetamol-induced animals.

**Keywords:** Acetaminophen, Black cumin, Black seeds, Cytochrome P450, Fennel flower, Ranunculaceae

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### INTRODUCTION

Reactive secondary metabolites of drug, xenobiotics, excessive alcohol consumption and some disease conditions are responsible for liver injury, from acute hepatitis indistinguishable of viral hepatitis to autoimmune syndromes, steatosis or rare chronic vascular syndromes, and from asymptomatic liver test abnormalities to acute liver failure. These compounds directly affect mitochondrial permeability transitional pore, mitochondrial respiratory chain, cytochromes P-450, and glutathione S-acyltransferases etc. Mostly carbon tetrachloride (CCl<sub>4</sub>), N-nitrosodiethylamine (NDEA), acetylaminofluorene (2-AAF), galactosamine, d-galactosamine/ lipopolysachharide (GalN/IPS), thioacetamide, antitubercular drugs, paracetamol, arsenic *et cetera* are usually used to induce experimental hepatotoxicity in laboratory animals. CCl<sub>4</sub>, D-GalN, paracetamol, and ethanol-induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and toxic chemicals. Paracetamol is commonly known to damage the centrilobular (zone III) hepatocytes, the primary sites of cytochrome P450. This drug influences MPTP opening (P 450 dependent) and causes mtDNA/cellular DNA damage [1, 2].

Efforts should also be directed toward the development of an abridged instrument for use in evaluating suspected drug-induced hepatotoxicity at the very beginning of the diagnosis and treatment process when

clinical decisions need to be made. The instrument chosen would enable a confident diagnosis to be made on the admission of the patient and treatment to be finetuned as further information is collected [3].

Silymarin (*Silybum marianum*) consists of four flavonolignan isomers namely--silybin, isosilybin, silydianin and silychristin. Among them, silybin being the most active and commonly used. Silymarin is orally absorbed and is excreted mainly through bile as sulphates and conjugates. This compound offers good protection in various toxic models of experimental liver diseases in laboratory animals [4]. Silymarin shows the ability to inhibit the enhanced ALT, AST, ALP, and platelet counts [5]. This drug has a good safety record and only rare case reports of gastrointestinal disturbances and allergic skin rashes have been published [6, 7].

It is clear that the medicinal plants play a significant role against various diseases. Different medicinal herbs and plants extracts have potent hepatoprotective activity in various animal models [8, 1]. *Nigella sativa*, a dicotyledon of the Ranunculaceae family, contains major phenolic compounds such as thymoquinone, dithymoquinone, thymohydroquinone, and thymol [9]. This work was aimed to investigate the hepato-nephroprotective activity of *Nigella sativa* (Ranunculaceae) oil on paracetamol-induced New Zealand rabbits (*Oryctolagus cuniculus*).

## MATERIALS AND METHODS

### *Nigella sativa* oil (NSO), standard drugs, and chemicals

*Nigella sativa* oil or NSO (Badan POM 093300411) in pharmaceutical preparation form was purchased from PT Habatussauda International, silymarin and paracetamol were obtained from Kimia Farma Bandung, Indonesia. A reagent kit for SGOT (Evogen), SGPT (Evogen), and gamma-GT (Evogen) were purchased from PT Rasani Karya Mandiri, South Tangerang, Indonesia.

### Animals

Fifteen New Zealand rabbits (*Oryctolagus cuniculus*) aged 3-4 mo (1.2-2.0 kg of BW), were purchased from Roemah Kelinci

Sumedang, West Java, Indonesia (<http://www.roemahkelinci.com/>). The rabbits were acclimatized for 7 d and treated according to the ethical standards of Universitas Padjadjaran

Health Research Ethics Committee. Standard feed (a mixture of soybean-curd and grass) and drink were given *ad libitum*. No vitamins were added in their feed.

### Experimental design

The animals were evaluated for their biochemical parameters (SGOT, SGPT, GGT, ureum-N, and creatinine) to obtain the baseline value. At the 8<sup>th</sup> day, the animals were divided into six groups, three in each group (2 males, 1 female), that were (table 1).

**Table 1: Animal group and the treatment for hepato-nephrotoxicity study**

Animal group	Treatment for 15 d		
	Water ml/kg of BW	Silymarin mg/kg of BW	NSO ml/kg of BW
I (normal control)	1.0	--	--
II (negative control)	1.0	--	--
III (positive control)	--	100	--
IV	--	--	0.5
V	--	--	1.0
VI	--	--	2.0

On the 16<sup>th</sup> day, after the rabbits were treated with silymarin or NSO for 15 d, rabbits in group II-VI were hepato-nephrotoxicity induced with paracetamol at a dose of 600 mg/kg of BW orally.

### Hepato-nephrotoxicity clinical measurements and qualitative histological study

At the 23<sup>rd</sup> day the animals were measured for their clinical biochemistry parameters (SGOT, SGPT, GGT, ureum-N, and creatinin), then they were anaesthetized by over dosage of solid CO<sub>2</sub> and sacrificed. The liver and kidney were immediately dissected out and transferred into Bouin solution (a mixture of picric acid, formalin, glacial acetic acid, and distilled water) for histological examination. The organs were then stained using H and E (hematoxyline and eosin), and the histological changes were observed under light-microscope and relevant findings were recorded on 40x magnification. This step was carried out at

Laboratory of Animal Biosystem, Department of Biology, Faculty of Mathematics and Natural Science, Universitas Padjadjaran, Jatinangor, West Java, Indonesia.

### RESULTS AND DISCUSSION

Paracetamol administration resulted in the elevation of SGOT, SGPT, and ureum-N levels of the animals, particularly in group II which was treated only with water and paracetamol (table 2 and 3). Pretreatment with silymarin (group III) and NSO (group IV-VI) significantly prevented the biochemical changes induced by paracetamol. Higher SD value was obtained due to the female hormonal-fluctuative results.

**Table 2: Influence of NSO on SGOT, SGPT, and GGT of paracetamol-induced hepato-nephrotoxicity models of New Zealand rabbits (*Oryctolagus cuniculus*)**

Animal group	SGOT (IU/l)		SGPT (IU/l)		GGT (IU/l)	
	Baseline	Day-23rd	Baseline	Day-23rd	Baseline	Day-23rd
I [H <sub>2</sub> O]	25±5.0	76±10.7	8±1.8	55±4.9	5±4.8	13±4.5
II [H <sub>2</sub> O+PCT]	22±0.8	92±33.5	9±1.6	73±27.8	6±2.7	14±1.9
III [SIL+PCT]	16±5.8	45±1.9	10±1.3	25±4.6	5±4.5	10±1.8
IV [NSO+PCT]	26±2.7	55±6.2	10±1.0	42±7.8	5±4.3	8±1.7
V [NSO+PCT]	18±5.1	40±13.8	11±1.2	37±13.2	5±5.5	8±1.2
VI [NSO+PCT]	21±19.0	32±16.9	9±1.8	35±11.5	6±4.3	7±4.3

Values are expressed as mean±SD, n = 3 rabbits (2 males, 1 female), PCT: paracetamol; SIL: silymarin; NSO: *Nigella sativa* oil

**Table 3: Influence of NSO on ureum-N and creatinine of paracetamol-induced hepato-nephrotoxicity models of New Zealand rabbits (*Oryctolagus cuniculus*)**

Animal group	Ureum-N (mg/dl)		Creatinin (mg/dl)	
	Baseline	Day-23rd	Baseline	Day-23rd
I [H <sub>2</sub> O]	23±14.1	41±11.2	0.6±0.2	0.7±0.1
II [H <sub>2</sub> O+PCT]	24±14.0	74±44.3	0.6±0.2	1.3±0.2
III [SIL+PCT]	22±10.4	61±9.2	0.8±0.5	1.1±0.5
IV [NSO+PCT]	21±9.0	47±12.4	1.0±0.9	1.2±0.5
V [NSO+PCT]	28±9.3	46±31.8	0.9±0.6	0.9±0.9
VI [NSO+PCT]	23±9.8	44±8.3	0.7±0.2	0.7±0.2

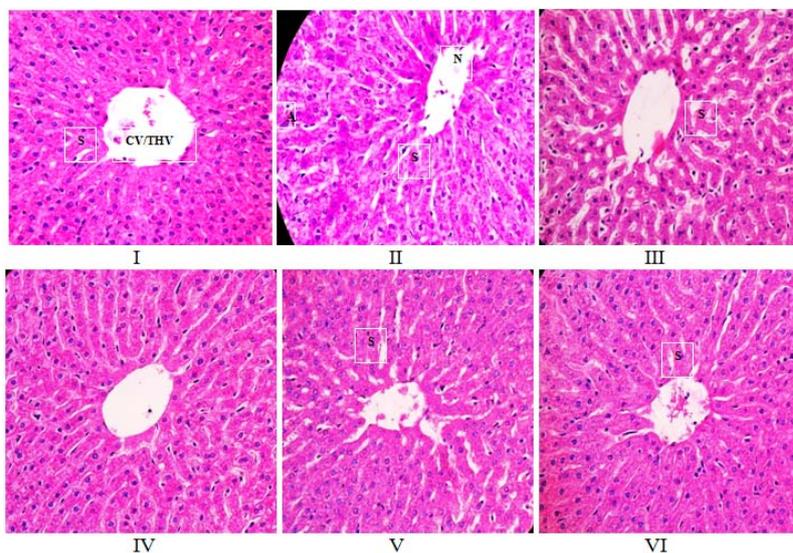
Values are expressed as mean±SD, n = 3 rabbits (2 males, 1 female), PCT: paracetamol; SIL: silymarin; NSO: *Nigella sativa* oil

Normal histology of the liver defines the clear shape of the THV/CV and sinusoids, as showed in fig.1-I. In the paracetamol-treated group (fig. 1-II), the THV/CV was normal, the sinusoids were dilated, whilst a necrosis (N) and mass apoptosis (A) were detected.

However, in the silymarin (fig. 1-III) group as well as in the lower and medium doses of NSO groups (fig. 1-IV and 1-V), the THV/CV was normal, but dilated sinusoids (S) were observed. In the highest

dose of NSO group (fig. 1-VI) the THV/CV and sinusoids were normal, regardless a local apoptosis and fat degeneration were detected.

Sinusoids are the channels through which blood flows from portal tracts to the hepatic venule. They are lined by endothelial cells and Kupffer cells, the latter of which is a specialized population belonging to the macrophage phagocytic system [10].

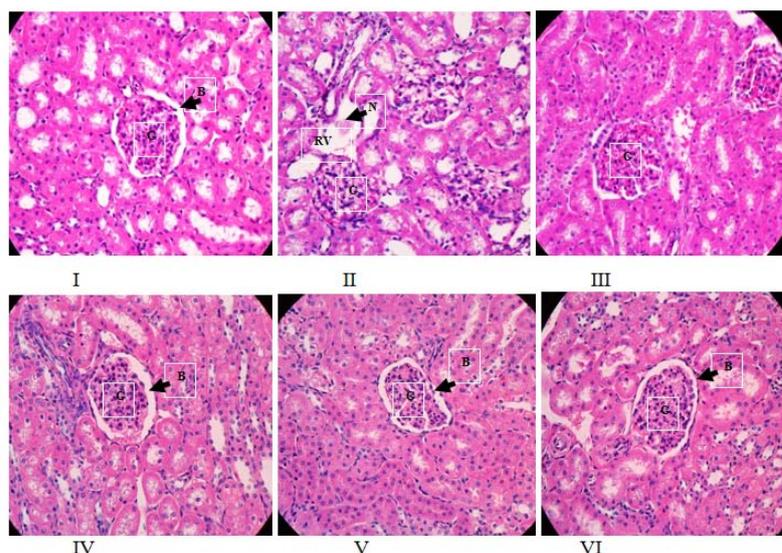


**Fig. 1: Effect of NSO on histological examination of the paracetamol-induced rabbit's liver (I: normal control group; II: negative control group; III: positive control group; IV: NSO 0.5 mg/kg of BW; V: NSO 1.0 mg/kg of BW; VI: NSO 2.0 mg/kg of BW). Magnification 40x**

The kidney architecture is composed of a large number of nephrons and hematopoiesis. Each nephron contains renal corpuscles and renal tubules. The renal corpuscles contain a vascular capillary glomerulus that is enclosed by Bowman capsule [11].

Normal histology of the kidney defines the clear shape of the Bowman capsule (B) and renal glomerulus shape (G) as showed in fig. 2-I. In the paracetamol-treated group (fig. 2-II) the Bowman capsule could not be detected although the glomerulus shape looked normal, moreover enlarged renal vein (RV) and local necrosis (N) of the kidney were found, which results in elevated SGOT, SGPT, and ureum-N on blood tests (table 2 and 3).

Our findings confirm the work of Payasi and colleagues (2010) that paracetamol (dose of 16 to 66 mg/kg body weight) has an effect on liver and kidney histological structure of mice. On the other hand, no major changes in physiological, haematological and biochemical parameters of the animals were observed [12]. Liver damage is always associated with cellular necrosis; a decrease in tissue lipid peroxidation and depletion in the tissue glutathione (GSH) levels. Moreover, serum levels of many biochemical markers like aspartate amino transaminase (AST), alanine aminotransaminase (ALT), SGOT, SGPT, total cholesterol, and total bilirubin (TB) are increased [13].



**Fig. 2: Effect of NSO on histological examination of the paracetamol-induced rabbit's kidney (I: normal control group; II: negative control group; III: positive control group; IV: NSO 0.5 mg/kg of BW; V: NSO 1.0 mg/kg of BW; VI: NSO 2.0 mg/kg of BW). Magnification 40x**

The silymarin-treated group (fig. 2-III) indicated an abnormality of the glomerulus shape and irregularity of Bowman capsule, compared to normal control group (fig. 2-I), although pretreatment with silymarin had been reported could repair the damage of liver and kidney induced by rifampicin [14].

The lowest dose of the NSO-treated group (fig. 2-IV) showed the normal shape of the renal glomerulus (G) and Bowman capsule (B), normal apoptosis. No necrosis was observed in the rabbit's kidney. Higher doses of NSO groups (fig. 2-IV and 2-V) indicated a normal glomerulus shape and Bowman capsule, and mass apoptosis and local necrosis.

In our work, *Nigella sativa* oil has been proven could maintain the normality of the liver and kidney of paracetamol-induced New Zealand rabbits (*Oryctolagus cuniculus*). This hepatonephroprotective activity is probably due to the presence of flavonoids, phenolic compounds, and polyphenols contained in *Nigella sativa* [9]. The protective action of thymoquinone, a phenolic compound of *Nigella sativa*, against tertbutyl hydroperoxide, a hepatotoxin, has been reported in rat hepatocytes [15]. Thymoquinone also showed other pharmacology activity, as it was predicted to have inhibitory activity on abnormal cell proliferation by modulating the activity of PTEN, a negative regulator of PI3K/AKT pathway [16]. Hepatoprotective properties of *Nigella sativa* in liver damage of experimental rats by reducing oxidative stress has also been reported [17].

Other hepatoprotective agents, e. g. baker yeast (*Saccharomyces cerevisiae*) was reported to preserve the structural integrity of hepatocellular membrane against sodium valproate-induced hepatotoxicity and oxidative stress [18]; whereas propolis could afford significant protection and therapy in the alleviation of hepatotoxicity induced by D-galactosamine and lipopolysaccharide in rats [19].

## CONCLUSION

In this study, *Nigella sativa* oil could maintain the normality of the THV/CV and sinusoids in the liver of paracetamol-induced New Zealand rabbits (*Oryctolagus cuniculus*). Normal glomerulus shape and Bowman capsule were also confirmed in the kidney of paracetamol-induced animals.

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## AUTHOR CONTRIBUTION

All authors contributed equally in the designing and performing this project.

## CONFLICT OF INTERESTS

Declared none

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