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Evaluation of Anti-ulcerogenic Activity in Oil Extract of Jintan Hitam (*Nigella sativa*) Against Ethanol Induced Gastric Ulcer in Mice (*Mus musculus*)

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Abstract: *Jintan hitam (Nigella sativa)* has been widely used in traditional medicine to treat several diseases, including gastric ulcer. However, there are no scientific data demonstrating anti-ulcerogenic activity conferred by use of *jintan hitam*. The present study aimed to evaluate the antiulcer properties of oil extract from *jintan hitam*. In the gastric ulcer induced by administration of 80% ethanol model, observation made for ulcer score and index, level of ulcer erosion, total amount of polymorphonuclear cells and morphological change of 80% ethanol induced gastric ulcer of mice. Research was conducted on laboratory in Faculty of Medicine Universitas Hasanuddin from April to September 2015, with post test only experimental group design. There were 40 mice used in this research, divided into five groups, each group consisted of 8 mice: negative control group, positive control group, and three different doses of oil extract (0.1, 0.2 and 0.3 ml per 20g bw of mice). Observation made for 4 and 7 days. The oil extract of *jintan hitam* showed significant differences on doses of 0.1 ml per 20g bw of mice given in 4 days, with $p = 0.034$ ($p<0.05$) based on score and index of gastric ulcer.

Keywords: Oil Extract of *Jintan Hitam*, Ulcer Score, Ulcer Index, Ulcer Erosion, Polymorphonuclear Cells, Morphological Change of Gastric

1. Introduction

Gastric ulcer is the damage that occurs from mucous layer to mucous muscular, round or ovoid shape with diameter > 5 mm, as the result of discontinuity of gastric mucous integrity [1, 2]. Gastric ulcer is believed to be due to an imbalance between aggressive and protective factors in the stomach, including infection of *Helicobacter pylori* [3, 4], non-steroid anti inflammatory drugs, alcohol consumption and stress condition [5, 6, 7].

Prevalence of gastric ulcer depends on social economic factors, demography, gender and age. There are 4 million (12%) cases of gastric ulcer in United States, with 500,000 new cases every year [1]. Data from Indonesia Ministry of Health in Riset Kesehatan Dasar 2007, showed that gastric ulcer was in the 14th place (1.7%) as the leading cause of death for people of all ages [8]. Data from endoscopic examination from 1615 patients with chronic dyspepsia at hospital in Makassar, showed 5% cases of gastric and duodenal ulcer [1].

The goals of treatment for gastric ulcer are to: relieve pain, heal the ulcer and prevent ulcer recurrence [1, 5, 6]. Drug

treatment of peptic ulcers is targeted at either counteracting aggressive factors (acid, pepsin, active oxidants, platelet aggravating factor, leukotriene, endothelins, bile or exogenous factors including NSAIDs) or stimulating the mucosal defenses (mucous, bicarbonate, normal blood flow, prostaglandins and nitric oxide) [9].

Several pharmaceutical drugs such as anticholinergic drugs, histamine H2-receptor antagonists, antacids, and more recently, proton-pump inhibitors have been employed in the management of peptic ulcers, but they provoke many adverse effects. Antacids which frequently used are calcium carbonate and sodium bicarbonate, in long term will cause Burnett syndrome (severe condition of hypercalcemia, hyperphosphatemia, with possibility to renal calcinosis and spreading into renal insufficiency). Sodium bicarbonate could induce systemic alkalosis [10]. Proton pump inhibitor (omeprazole) may cause abdominal pain, nausea, constipation and diarrhea. H2-receptor antagonist (cimetidine) may cause loss of libido and gynaecomastia [7]. Currently, there is no cost-effective treatment that meets all these goals. Hence efforts are on to find a suitable treatment from natural product sources [10, 11, 12].

Indonesia has abundant natural resources, second place behind Brazil. However they are still not utilized to the maximum level. Use of herbal medicine is still based on empirical information or passed down from generation to generation without scientific research [13]. World Health Organization has been developing strategy for traditional medicine, to support potential traditional medicine and to promote safe and effective traditional medicine, through regulation, research and integration practice of traditional medicine in health system [14]. Ministry of Health has been regulating traditional health services into empirical, complementary and integrated system [15].

Nigella sativa or *Habbah Al Sauda* is known as *jintan hitam* in Indonesian. *Nigella sativa* is native to Southern Europe, North Africa and Southwest Asia and it is cultivated in many countries in the world like: Middle Eastern Mediterranean region, South Europe, India, Pakistan, Syria, Turkey and Saudi Arabia. The seeds of *Nigella sativa* have been widely used in the treatment of various diseases such as bronchitis, asthma, diarrhea, rheumatism and skin disorder. *Nigella sativa* has been extensively studied for its therapeutic potential as diuretic, antihypertensive, anti diabetic, anticancer and immunomodulatory, analgesic, antimicrobial, anthelmintic, analgesics and anti-inflammatory, spasmolytic, bronchodilator, gastro protective, hepatoprotective, renal protective and antioxidant properties [16, 17, 18, 19].

One such therapeutically important component of *Nigella sativa* is Thymoquinone (2-isopropyl-5-methyl-1,4-benzoquinone) (THQ). Botanically, THQ is found in the seeds of *Nigella sativa* containing up to 30-48% of THQ along with p-cymene (7-15%), carvacrol (6-12%), 4-terpineol (2-7%), t-anethole (1-4%) and sesquiterpene longifolene (1-8%) [20]. Thymoquinone as component from oil extract of *Nigella sativa*, has prominent molecular target as anti-oxidant and anti-inflammation. Anti-oxidant effects are developed through scavenging action, induction of anti-oxidant enzyme and increasing mitochondria function. Meanwhile thymoquinone has an effect as anti-inflammation by reduction IL-1β, TNF-α, and 5-LOX [21].

Study about gastro protective effects from oil extract of *Nigella sativa* to ethanol induced gastric ulcer showed that thymoquinone could accelerate healing wound process, increased gastric glutathione content, activity of superoxide dismutase and glutathione S transferase enzyme [22]. *Nigella sativa* has been proved as effective as cimetidine for healing gastric ulcer, and could be given to patients in general practices [23]. Another study about hemorrhagic and edema, at histological specimen of alcohol induced gastric ulcer in rats, showed improvement with increasing in epithel regeneration on gastric mucosa, after given *Nigella sativa* oil extract [24]. Study of 88 dyspepsia patients without gastric ulcer showed *Nigella sativa* with omeprazole providing as effective as combination therapy (clarithromycin, amoxicillin and omeprazole) for *Helicobacter pylori* eradication [25].

A large percentage of world population, especially in the developing countries, relies on natural remedies to treat a variety of diseases. On that ground, a large number of spices and herbs have been evaluated by various researchers for their anti-ulcer effects. Since the comprehensive integrated and standardized study for *jintan hitam* was not held yet, *Jintan hitam* was considered only for health supplement [26]. Considering the need to search for resources for the treatment of gastric ulcers and growth in scientific research about medicinal plants, the present study aimed to evaluate the anti-ulcer properties of oil extract of *jintan hitam*.

2. Material and Methods

This post test controlled group design of experiment study which was conducted in Animal Laboratory and Laboratory of Pathology Anatomy, Faculty of Medicine, Hasanuddin University, and laboratory in Faculty of Pharmacy, Universitas Muslim Indonesia, from April to September 2015. *Jintan hitam* seed that was examined in this study was bought from traditional market in Makassar. Extraction process was done to get oil extract from *jintan hitam* seed. Preliminary study was held to obtain optimal gastric ulcer model in mice. The study protocol was approved by the Research Ethics Committee on Faculty of Medicine, Hasanuddin University number 1770/ H4.8.4.5.31/ PP36-KOMETIK/ 2015.

This study was using 40 male mice (*Mus musculus*), 5-7 weeks old, 20-35 g bw, in healthy condition. Group (1) consist of eight mice which didn't receive anything (negative control), other groups received 80% ethanol 0.1 ml per 20g bw for 2 days. Group (2) consist of three mice after received ethanol for 2 days, was given regular food for 4 days and sacrificed on day 5 (positive control). Group (3) consist of five mice after received ethanol for 2 days, was given regular food for 7 days and sacrificed on day 8 (positive control). Group (4) to (6) consist of three mice each group, after received ethanol for 2 days, was given oil extract of *jintan*

hitam 0.1, 0.2 and 0.3 ml per 20g bw, once a day for 4 days and sacrificed on day 5. Group (7) to (9) consist of five mice each group, after received ethanol for 2 days, was given oil extract of *jintan hitam* 0.1, 0.2 and 0.3 ml per 20g bw, once a day for 7 days and sacrificed on day 8.

All mice were sacrificed, stomach were isolated and then dissected out through opening along the gaster curvature pyloric section, inspected internally for ulcer score, ulcer index and level of ulcer erosion. All specimens were processed by histokinet for making paraffin block and Hematoxyllin Eosin and Giemsa coloring. All specimens inspected for polymorphonuclear cells and morphological change of gastric mucous. Results as the statistical significance was determined by Chi-Square, Mann-Whitney and t test, with the minimum level if significance set at $p < 0.05$.

3. Results

Table 1. *Characteristics of Sample after Treatment (n = 40).*

Characteristics		Value
Ulcer Score	Mean + SD	0.45 + 0.85
	Median	0
	Maximum-minimum	0-3
Ulcer Index	Mean + SD	0.12 + 0.22
	Median	0
	Maximum-minimum	0-0.67
PMN Leucocyte (/HPF)	Mean + SD	10.93 + 13.29
	Median	7.5
	Maximum-minimum	0-53
Erosion Level (n(%))	No lesion	8 (20)
	Small	22 (55)
	Medium	8 (20)
	Large	2 (5)
Morphological Change (n(%))	Yes	19 (47.5)
	No	21 (52.5)

Note : SD = standard deviation, PMN = polymorphonuclear, HPF = high power field

Table 2. *Ulcer Score on Day-4 for Control and Treatment Group.*

	Group				
	Negative Control	Positive Control	Jintan 0.1 ml	Jintan 0.2 ml	Jintan 0.3 ml
N	3	3	3	3	3
Mean + SD	0	2	0.33 + 0.58	0.33 + 0.58	0.33 + 0.58
Median	0	2	0	0	0
Minimum	0	2	0	0	0
Maximum	0	2	1	1	1

Note: SD = standard deviation

P value for Mann-Whitney test
a K- vs. K+ = 0.002
b K- vs. P1 = 0.317
c K- vs. P2 = 0.317
d K- vs. P3 = 0.317
e K+ vs. P1 = 0.034*
f K+ vs. P2 = 0.034*
g K+ vs. P3 = 0.034*
h P1 vs. P2 = 1.000
i P1 vs. P3 = 1.000
j P2 vs. P3 = 1.000

Based data on table 2, Mann-Whitney test in treatment group with 0.1, 0.2 and 0.3 ml oil extract of *jintan hitam* showed p=0.034 (p<0.05) with significance differences of ulcer score on day-4 compared to positive control group.

Table 3. *Ulcer Score on Day-7 for Control and Treatment Group.*

	Group				
	Negative Control	Positive Control	Jintan 0.1 ml	Jintan 0.2 ml	Jintan 0.3 ml
N	5	5	5	5	5
Mean + SD	0	1.8 + 1.1	0	0	0
Median	0	2	0	0	0
Minimum	0	0	0	0	0
Maximum	0	3	0	0	0

Note : SD = standard deviation

P value for t-test
a K- vs. K+ = 0.006
b K- vs. P1 = 1.000
c K- vs. P2 = 1.000
d K- vs. P3 = 1.000
e K+ vs. P1 = 0.006*
f K+ vs. P2 = 0.006*
g K+ vs. P3 = 0.006*
h P1 vs. P2 = 1.000
i P1 vs. P3 = 1.000
j P2 vs. P3 = 1.000

Based data on table 3, t-test in treatment group with 0.1, 0.2 and 0.3 ml oil extract of *jintan hitam* showed p=0.006 (p<0.05) with significance differences of ulcer score on day-7 compared to positive control group.

Table 4. *Ulcer Index on Day-4 for Control and Treatment Group.*

	Group				
	Negative Control	Positive Control	Jintan 0.1 ml	Jintan 0.2 ml	Jintan 0.3 ml
N	3	3	3	3	3
Mean + SD	0	0.67	0.11 + 0.19	0.11 + 0.19	0.11 + 0.19
Median	0	0.67	0	0	0
Minimum	0	0.67	0	0	0
Maximum	0	0.67	0.33	0.33	0.33

Note: SD = standard deviation

P value for Mann-Whitney test
a K- vs. K+ = 0.025 *
b K- vs. P1 = 0.317
c K- vs. P2 = 0.317
d K- vs. P3 = 0.317
e K+ vs. P1 = 0.034*
f K+ vs. P2 = 0.034*
g K+ vs. P3 = 0.034*
h P1 vs. P2 = 1.000
i P1 vs. P3 = 1.000
j P2 vs. P3 = 1.000

Based data on table 4, Mann-Whitney test in treatment group with 0.1, 0.2 and 0.3 ml oil extract of *jintan hitam* showed p=0.034 (p<0.05) with significance differences of ulcer index on day-4 compared to positive control group.

Table 5. *Ulcer Index on Day-7 for Control and Treatment Group.*

	Kelompok				
	Negative Control	**Positive Control**	**Jintan 0.1 ml**	**Jintan 0.2 ml**	**Jintan 0.3 ml**
N	5	5	5	5	5
Mean + SD	0	0.36 + 0.22	0	0	0
Median	0	0.4	0	0	0
Minimum	0	0	0	0	0
Maximum	0	0.6	0	0	0

Note : SD = standard deviation

P value for t-test

- K- vs. K+ = 0.021
- K- vs. P1 = 1.000
- K- vs. P2 = 1.000
- K- vs. P3 = 1.000
- K+ vs. P1 = 0.021*
- K+ vs. P2 = 0.021*
- K+ vs. P3 = 0.021*
- P1 vs. P2 = 1.000
- P1 vs. P3 = 1.000
- P2 vs. P3 = 1.000

Based data on table 5, t-test in treatment group with 0.1, 0.2 and 0.3 ml oil extract of *jintan hitam* showed p=0.006 ($p<0.05$) with significance differences of ulcer index on day-7 compared to positive control group.

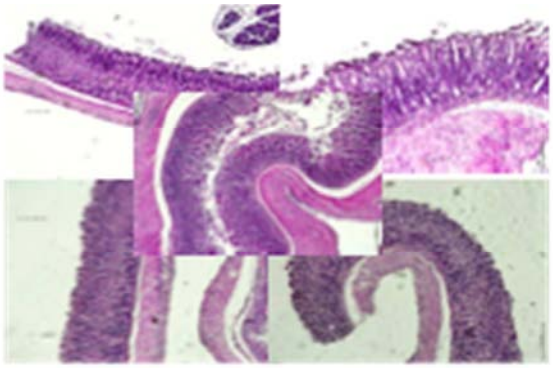
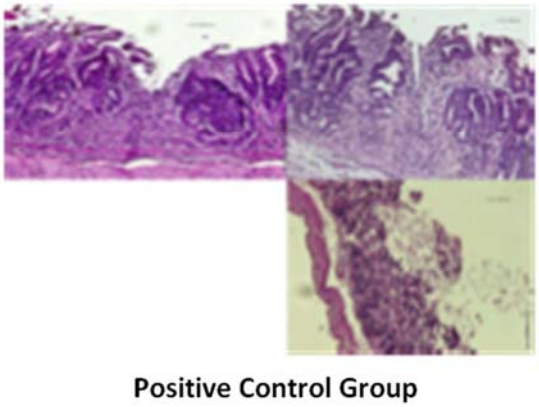


Fig. 1. *Morphological Change on Negative Control Group (objective 20x).*



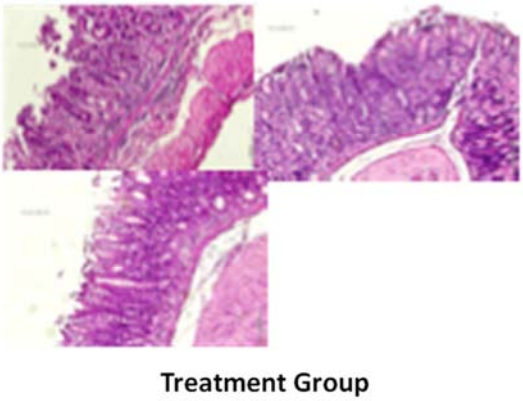


Fig. 2. *Morphological Change on Positive Control and Treatment Group on Day-4 (objective 20x).*

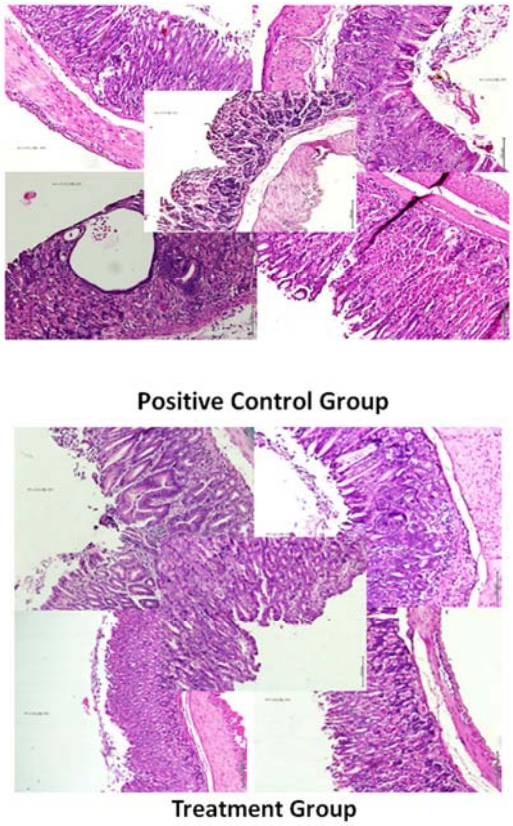


Fig. 3. *Morphological Change on Positive Control and Treatment Group on Day-7 (objective 20x).*

4. Discussion

Alcohol is absorbed rapidly through the bloodstream from the stomach and intestinal tract. High concentrations of ethanol induce vascular endothelium injury of the gastric mucosa, which become edematous, and congestive, present point and scattered bleeding lesions, focal hemorrhage, necrosis, and giant deep ulcers were visible. Principal cells and parietal cells become swollen and diminished. These cells are rich in mitochondria, an easily injured organelle. Evidences showed that ATPase decreased in ethanol-induced acute injury, and the lack of ATP may lead to metabolic acidosis, cellular edema, intracellular calcium overload, and

further damage to gastric mucosa cells. Alcohol exposure affects the mitochondrial structure which becomes swollen and disaggregated. Therefore, it was proposed that enlargement of the mitochondria is an adaptive process by which cells attempt to decrease the intracellular amount of ROS when they are subjected to oxidative stress. Gastric mucosa is rich in protein sulfhydryl groups, which may be the target of ROS, thus contributing to mucosal injury [27].

Ethanol has effects on mitochondrial function. Alcohol is metabolized to acetaldehyde by the cytosolic enzyme alcohol dehydrogenase (ADH). Mitochondrial aldehyde dehydrogenase 2 (ALDH2) converts acetaldehyde to acetate. When this enzyme is malfunctioning, acetaldehyde increases and damages the electron transport complexes (CI-CIV) leading over production of reactive oxygen species (ROS). Additionally, oxidative stress affects the permeability of the outer/inner mitochondrial membranes (OMM/IMM) promoting opening of the permeability transition pore (PTP). When the mitochondrial permeability transition is extensive, it promotes the mitochondrial swelling and permits the cytochrome c release (Cyt c), caspase activation and DNA fragmentation, leading the programmed cell death or apoptosis [28, 29].

Result of the study in treatment group with 0.1, 0.2 and 0.3 ml oil extract of *jintan hitam,* showed $p=0.034$ ($p<0.05$) on day-4 and $p=0.006$ ($p<0.05$) on day-7, with significance differences of ulcer score and ulcer index, compared to positive control group. The result was similar with study using black cumin extract that showed healing effects and decreasing of gastric ulcer index. Nigella sativa is equally effective in healing of gastric ulcer as is cimetidine, therefore it suggest the use of the Nigella sativa in the therapy of gastric ulcer in routine practice [23]. Other study using 200 and 400 mg per kg bw black cumin extract to rats, showing decrease of ulcer index and mild distortion in gaster mucous [12]. Another study using 500 mg per kg bw black cumin to rats for 5 days, showing ulcer index better than Ranitidine. It proved black cumin has potential gastro protective action [30]. The result was similar to study comparing black cumin and sodium diclofenac as it showed no significance differences between control and treatment group with black cumin or sodium diclofenac, for total leucocyte count and differential leucocyte count [31]. Different result came from study using thymoquinone as it showed reduction of neutrophil invasion with decreasing MPO (myeloperoxidase) activity on gastric injury model [32].

Initiation healing process of gastric ulcer started at the third day after ulcerogenic induction which in this study was using 80% ethanol. First step was re-epitelization; second step was formation, migration and reconstruction of the glands; third step was angiogenesis and forth step was remodeling [33]. Healing process of gastric ulcer including several processes in gaster mucous e.g. congestive, hemorrhagic, edema, necrosis, inflammation, erosion, ulceration and dysplastic change [16]. Evaluation for healing process in clinical setting was based on visual endoscopy, but in this study was based on microscopic evaluation and gastric ulcer determination. Microscopic evaluation showed gastric glands dilatation, increase of connective tissue, increase of micro vascularization and recovery of sensory nerve. It could be the base for evaluating quality of healing process of gastric ulcer [33].

5. Conclusion

Treatment group with 0.1, 0.2 and 0.3 ml oil extract of *jintan hitam* showed $p=0.034$ ($p<0.05$) on day-4 and $p=0.006$ ($p<0.05$) on day-7, with significance differences of ulcer score and ulcer index compared to positive control group. Further research is needed to evaluate changing process in gastric mucous in day-1 until day-6, based on ulcer score, ulcer index, erosion level and amount of PMN leucocyte.

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