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Research Article

Kersen Leaf (MuntingiaCalabura L.) Extract Prevents Gastric Damage of Wistar Rats Exposed to 40% Ethanol

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ABSTRACT

Alcohol abuse consumption inducesoxidative stress and resulting the gastric mucosal injury. Kersen Leaf are known asantioxidants. This study aimed to know the effect ofKersen leaf extract to preventthemucosal stomach damage in alcoholic induced Wistar Rats. This study was experimental laboratory witha Post test only group design approach. The sample consisted of 24 male wistar rats divided into 4 groups, namely K- given placebo aquadest 1.8ml/ 200g/ day, K+ given ethanol 40% dose 1.8ml/ 200g /, P1 and P2 were rats induced to 40% alcohol and treated with Kersen leaf extract (500 mg/ kg body weight for P1 and 750 mg/ kg body weight for P2), 60 minutes after administration of alcohol. Thegastric damage score was measured based onBarthel-Manja score. The mean difference in the level of gastric mucosa damage between groups was analyzed by One Way ANOVA Test and Post hoc Lsd Test. The average ofgastric damage score were;K- = 0.44, K+ = 1.76, P1=0.76 and P2=0.44 ($p<0.001$). Differences in degrees of gastric mucosal damage at K+, P1, P2 compared to K- respectively (ρ: <0.001); (ρ: 0.143); (ρ: 1.000). The administration of Kersen leaf extract at a dose of 500mg/ Kgand 750mg/ Kgis able to prevent gastric mucosa damage induced by 40% ethanol.

Keywords : ethanol, Kersen leaf extract, gastric mucosal histopatology.

INTRODUCTION

Based on the Global Status Report on Alcohol and Health 2018, about 2,084,649 people of Indonesia experienced health problems due to alcohol consumption. At least1,824,068 people of Indonesia become alcoholic abuse.Alcohol abuse caused about 1.7 million deaths from non-communicable diseases in 2016, including 1.2 million deaths caused by digestive and cardiovascular diseases (WHO, 2018). The danger of consuming alcohol is included in the top five risk factors for disease, disability and death all over the world (Baan et al., 2007).

Alcohol is absorbed in the stomach without changing its component after ingested. Alcohol then spreads to all tissues and body fluids according to levels in the blood (Tritama,

2015).This can induce weakening the defense of the gastric mucosa and inducing gastric mucosal injuries including gastritis, ulcers and possibly developing malignancy of stomach (Tritama, 2015).

Previous studies have reported inflammatory processes and oxidative stress responsible for gastric mucosal injury (Li et al., 2016). The inflammatory process is characterized by pro-inflammatory cytokines such as Tumor necrosis factor-alpha (TNF-α) and Interleukin-6 (IL-6) (Rozza et al., 2014). TNF-α as the first line of cytokines whichinduce the production of Reactive Oxygen Species (ROS) and as genes that are responsive to oxidative stress. IL-6 is a proinflammatory cytokine secreted by T cells and macrophages to stimulate the immune response, that causes local tissue damage. Gastric mucosal injury is also caused by oxidative stress, which is an imbalance between the amount of ROS and antioxidants in the body (Rahal et al., 2014). The main source of ROS in the ethanol-induced stomach is neutrophil-activated infiltration which further stimulates the release of pro-oxidative enzymes and free radicals which then cause oxidative stress (Amirshahrokhi&Khalili, 2015).

Under the oxidative stress condition, an imbalance between the production of ROS and antioxidants results in theabnormal oxidation-reduction chain.This condition can be overcome by exogenous and endogenous antioxidant.Inside the body will neutralize free radicals by releasing SOD (Supeoxid dismutase) and Gluthathuione (GSH)in the stomach. While from outside the body, there are some herbs, foods which contain antioxidants used as chain-breaking antioxidants, like vitamin C, vitamin E, beta carotene, and flavonoid classes (Kurniati, 2017)

Kersen Leaf have strong antioxidant activity because they contain compounds such as flavonoids, tannins, polyphenols, and saponins. The nutritional content of cherry leaves in each 100 grams includes water, protein, fat, fiber, calcium, phosphorus, carotene, vitamins B1, B2, B3 and C (Nugroho, 2012). Kersen leave is one source of natural antioxidants which easily cultivated in Indonesia. The previous studies stated, Kersen leaf extract with a dose level of 500mg/ Kgbody weightwas able to provide a significant defense against the integrity of the esophageal mucosa ($p = 0.000$).This study aimed to know the effect of Kersen leaf extract to prevent the mucosal stomach damage in alcoholic induced Wistar Rats.

METHOD

The research were conducted at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, UniversitasNegri Semarang, the Anatomical Pathology Laboratory of the Faculty of Medicine, University of Muhammadiyah Semarang and Roemani Hospital Muhammadiyah Semarang. The research was True Experimental with Post Test Only Group Design,performed on male Wistar rats aged 6-8 weeks, weighs 150-200 grams,healthy and has no anatomical abnormalities. Rats were obtained from the Biology laboratory at the Faculty of Mathematics and Natural Sciences, State University of Semarang. The sample consisted of 24 male wistar rats divided

into 4 randomized groups. The group consisted of K- given placebo aquadest 1.8 ml/ 200g/ day, K+ given ethanol 40% dose 1.8 ml/ 200g/, P1 was given 40% ethanol after 60 minutes of administration of 500mg/ Kgbody weightKersen leaf extract and P2 was given ethanol 40% after 60 minutes of administration of 750mg/ Kgbody weightKersen leaf extract (Ninditya et al., 2016). Treatment for 30 days, then the rats were terminated on the 31st day and carried out the process of sampling the stomach organs of the wistar rats and then made histological preparations with the paraffin block method by Hematoxilin eosin (HE) staining. Observation of the histopathologica feature of the wistar rat's stomach was carried out using a trinocular microscope with a magnification of 100x in 5 visual fields for each gastric sample of the Wistar rat. The observation was carried out by a specialist in Pathology Anatomy. Gastric mucosal damage was measured acording the score with Barthel-Manja criteria (Irramah et al., 2017). The mean degree of gastric mucosal damage was analyzed using the One way ANOVA test and the Post hoc Lsd Test.

The extract ingridients came from old Kersen Leaf, not exposed to pests and undamaged. Kersen Leaf were obtained from Sidomukti Village, BandunganSubdistrict, Semarang Regency with a dry weight of two kilograms. The dried leaves were mashed with a grinding machine. Kersen Leaf powder weighed 150 gram and then put into a one liter erlenmeyer size. Pour 600 ml of ethanol 70% into an erlenmeyer, homogenized with an incubator shaker for 60 minutes or until completely mixed. Kersen Leaf filtrate was filtered with filter paper and repeated 5 times. An evaporation process was carried out to separate the ethanol solution from the active ingredients present in the extract. The filtering results were entered into Erlenmeyer. The filtering results were concentrated with a rotary evaporator at a temperature of 65-70^{o} C for two hours.[19] The next stage of Kersen Leaf was examined thequalitative and quantitative test to determine the type of flavonoids compound and the levels of the dominant flavonoids. The examination was conducted at the Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, UniversitasNegri Semarang

This research was conductedbasedethical clearance from the Medical Research Ethics Commission of the Faculty of Medicine, UniversitasNegri Semarang, published number 093/ EC/ FK/ 2019.

RESULTS

The extraction of 150 gramskersen leaf powder mixed with600 ml of 97% ethanolyieldedconcentrate extract about16,404/ 100gr.The results of phytochemical analysis of cherry leaf extract are shown in table 1. Quantitative test found that the dominant content of the extract was quecertin as much as 1.5 mg/16,404 gr.

Table 1. Results of Phytochemical Analysis of Kersen Leaf Extract

No	Testing Parameter	Results	Notes
1.	Saponin	+	Formed foam that does not disappear for more than 1 minute
2.	Flavonoids	+	Formed a red color
3.	Tannin	+	Formed a Greenish black color
4.	Alkaloids	+	Formed a White sediment
5.	Phenolic	+	Formed a Green-purple color
6.	Terpenoids	-	No red/ pink color formed
7.	Steroids	+	Formed a Blue/ green color
8.	Flavonoids	1.5mg Querstein	

The effect of kersen leaf extract on the histopathological picture of the stomach of the 40 % etanol induced wistar rat are shown in table 2.Mucosal damage scores using the BarthelManja Index arescore 0: There were no pathologic changes, score 1: epithelial desquamation, score 2: epithelial surface erosion, score 3: epithelial ulceration

Table 2. Mean and Different Tests for Stomach Mucosal Damage Score

Group	Mean ± SD	One way annova (ϱ value)	Post hoc LSD (ϱ value)			
			K-	K+	P1	P2
K–	0.44 ± 0.46	<0.001*	-	<0.001**	0.143	1.000
K+	1.76 ± 0.17		<0.001**	-	<0.001**	<0.001**
P1	0.76 ± 0.38		0.143	<0.001**	-	0.143
P2	0.44 ± 0.22		1.000	<0.001**	0.143	-

Table 2. One-star (*) using the One way anova Test ϱ <0.05 and two-star (**) using the post hoc Lsd Test (ϱ <0.05); K-ie negative control group, given a placebo aquadest 1.8 ml/ 200g/ day without being given a 40% extract of Kersen Leaf or ethanol. K+ is a positive control group, given ethanol without administration of kersen leaf extract. P1 is treatment group 1, given kersen leaf

extract with a dose of 500mg/ Kgbody weight and ethanol 40%. P2 is Treatment group 2, given kersen leaf extract dose of 750mg/ Kgbody weight and ethanol 40%.

The degree of gastric mucosa damage among four groups was analyzed by the One way ANOVA test (Table 2). The results found significant differences of mucousal damage grade from all groups (ρ<0.001).The Post hoc test continued with the Least significant differences (Lsd) test. The gastric mucousal damage score in the K+ compared to K- was signficantly different (ρ: <0.001).The treated group with kersen leaf extract with the dose of 500mg/ Kgbody weight(P1) and a dose of 750mg/ Kgbody weight (P2), the score of gastric mucosaldamage not signicantly differentwith normal group (K-)(ρ: 0.143) and (ρ:1.000).

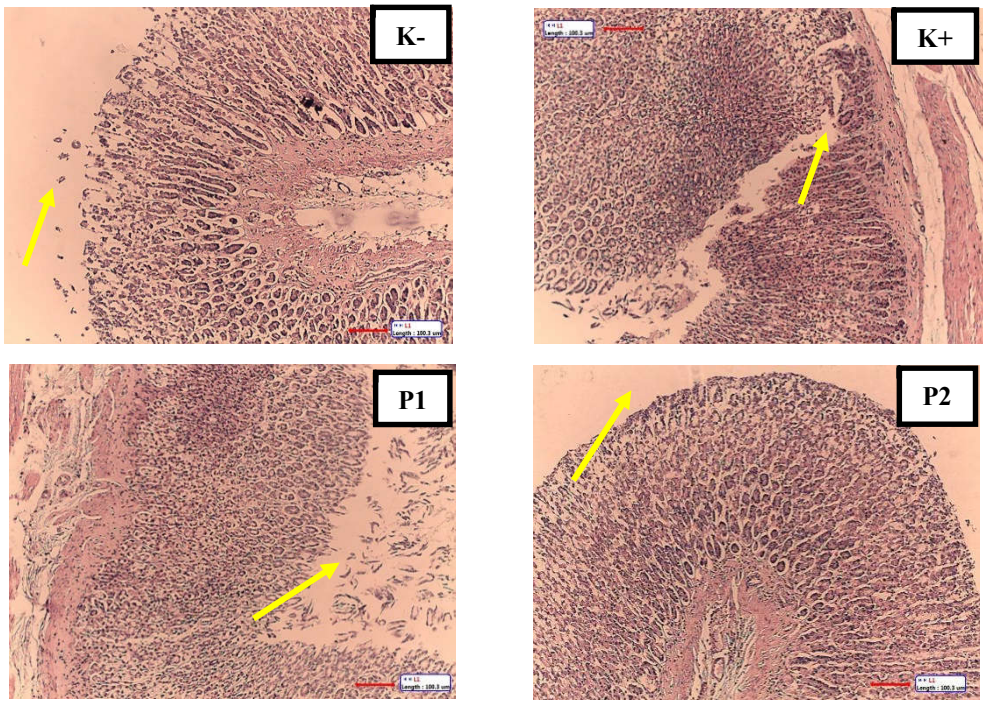

Figure 1. Histopathology picture of gastric mucosa with magnification 100x. Histopathology picture of the stomach among groups. From the picture it can be seen the average score of each group. The negative control group (K-) showed intact epithelium and minimum epithelial desquamation with a mean damage of 0.44. Positive control group (K+) seen mucosal damage (epithelial erosion) but the damage has not reached the submucosal layer with an average damage of 1.76. Treatment group 1 (P1) appears to be a dominant epithelial desquamation with a mean damage of 0.76. Treatment group 2 (P2) appeared to be dominated by intact epithelium with a clean surrounding field of view (mean damage 0.44)

DISCUSSION

Treatment ofKersen Leaf (Muntingiacalabura L.) extractin wistar rats which induced by 40% ethanol for 30 days, it was found that gastric mucosa damage in the K+ group was significantly different compared to the K- group. The degree of damaged mucosal damage in P1 and P2 was not significantly different from K-.

In the Normal rats (K-) given placebo aquadest 1.8 ml/ 200g/ Kgbody weight showed a normal histological picture of the stomach, but there are somedesquamation appearence. Desquamation found in the negative control group due to the release of epithelial elements as a defense response of the tissue against irritation or physiological process (Sherwood, 2014). Normal gastric mucosal desquamation occure withoutmucosal triggering factors such as 40% ethanol given to other groups. This is relevant to the previous study that administration of aquadest to gastric rats did not cause lesions or damage to the gastric epithelial mucosa of rats (Dewi et al., 2013).

The gastric mucosal damage scorefrom K- group to K+ gave significantly different results. In the positive control group histopatholgical feature, showdesquamation, erosion damage almost reached the entire thickness of epithelial mucosa that could be included in the category of epithelial ulcers. Alcohol metabolism into the body causes an imbalance in the production of ROS and antioxidants which will cause oxidative stress. This oxidative stress will affect the permeability of cell membranes. It induce translocation from pro-apoptotic factorswhich activates apoptotic enzymes and triggers the death of cells (Dorokhov et al., 2015).These results are suitable with previous studies that the administration of ethanol levels of 43% for five consecutive days changes in gastric histopathology in the form of erosion, inflammatory cells, intestinal metaphase and blood vessel dilation (Kololu, 2014). Previous research also stated that 50% ethanol administration could cause damage to gastric mucosa integrity and causespeptic ulcer (Kholifaturrokhmah&Purnawati, 2016).

The results of treatment in the form of administration of Kersen leaf extract at a dose of 500mg/ Kgbody weight and 750mg/ Kgbody weight in wistar rats exposed to 40% ethanol, did not show any differences in the degree of gastric damage when compared with normal mice (K-). This study is relevant to previous studies that the administration of extract before ethanol prevent gastric mucosal injury in rats by decrease the neutrophil infiltration of gastric mucosa (Abdulla et al., 2010).Previous studies regarding protective measures by Kersen extract against ethanol-induced gastric mucosal injury as shown by reduction or inhibition of gastric ulcer areas and increased production of gastric mucous and decreased gastric acidity (Ibrahim et al., 2014).

The results indicate that the administration of increasing doses of 500mg/ Kgbody weight and 750mg/ Kgbody weightshows dose dependent effect in preventinggastric mucosa damage. Kersen Leaf extract gives a gastro protective effect shown by a decrease in the average score of mucosal damage at doses of 500mg/ Kgbody weight and 750mg/ Kgbody weight. At a dose of

500mg/ Kgbody weight it appears that there is a dominance of epithelial desquamation while 750mg/ Kgbody weightdoses appear to dominate intact mucosa and the area around the mucosal appear clean. These results are relevant to previous studies regarding the increased dose of 250mg/ Kgbody weight and 500mg/ BW Kersen leaf extract in absolute ethanol-induced rats showed an increased effect of preventing gastric mucosal damage marked by mild mucosal damage with the presence of edema and leukocyte infiltration in the submucosa layer. Whereas at a dose of 500 mg/ kg showed mild damage to the epithelial mucosa without edema and leukocyte infiltration in the submucosa layer (Ibrahim et al., 2014).

The phytochemical test, the Kersen leaf extract positively contained some active compoundsofflavonoids. The main content of the extract used in this study is quesertin, which captures its action point as an antioxidant and anti-inflammatory to the gastric mucosa whichprevent damage to the gastric mucosaof rat exposed to 40% ethanol. Quersetin is the most important group of flavonoids as antioxidant compounds. The mechanism of action of antioxidants is divided into two functions. Antioxidant action as a giver of hydrogen atoms or often referred to as primary antioxidants. These compounds can give hydrogen atoms quickly to lipid radicals or change them to a more stable form. The other action is a secondary function of antioxidants. It slows the rate of autoxidation by various mechanisms beyond the mechanism of autoxidation chain breaking by converting lipid radicals to a more stable form. Each group of flavonoids from the extracthas a good capacity as an antioxidant. Flavon groups and catechins which have the highest activity to prevent the body from attacking free radicals (Simanjuntak, 2012).

Quersetin in addition to being an antioxidant can function as an anti-inflammatory. Cyclooxygenase and liposigenase play an important role in inflammatory mediators. The oxidation of arachidonic acid that releases both substances starts the inflammatory response. Neutropyl contains liposigenase which produces chemotactic compounds from arachidonic acid to release cytokines. The presence of phenolic compounds (flavonoids) can inhibit both the cyclooxygenase and liposigenase pathways. Quersetin inhibits the activity of both pathways by reducing the formation of inflammatory metabolites (simanjuntak, 2012). Quersetin is a free radical scavenger that efficiently reduces histamine secretion from mast cells which is then able to protect the gastric mucosa from ulcerogenesis (Jannah&Zuraidah, 2016).

This researchonly conducts the Post test control group design research design, so the further researchwill be better usingPre test and Post test control group design,so the condition of the stomach organs can be identifiedbefore the treatment, further research should explore the content of pure active substances and standardized dose of Kersen leaf extract. Acute and chronic toxicity tests on administration of Kersen leaf extract also needed

CONCLUSION

The administration of Kersen leaf extract at a dose of 500mg/ Kgbody weight and 750mg/ Kgbody weight is able to prevent gastric mucosa damage induced by 40% ethanol.

REFERENCES

Abdulla, M.A., Ahmed, K.A.A., Al-Bayati, F.H, Masood, Y. (2010). Gastroprotective effect of Phyllantusniruri leaf extract against ethanol-induced gastric mucosal injury in rats. Afri J Pharm Pharmacol., 4, 226-30.

Adawiah, Sukandar, D., Muawanah, A. (2015). Antioksidan dan kandungan komponen bioaktif sari buah Namnam. *Jurnalkimia VALENSI,* 1(2), 130-6.

Amirshahrokhi, K., Khalili, A.R. (2015). The effect of thalidomide on ethanol-induced gastric mucosal damage in rats: involvement of inflammatory cytokines and nitric oxide. Chem. *Biol Interact*, 225, 63–9.

Baan, R., Straif, K., Grosse, Y., Secretan, B., El, G.F., Bouvard, V. (2007). Carcinogenicity of alcoholic beverages. *The Lancet Oncology,* 8(4), 292-93.

Chang, X.Y., Luo, F., Jiang, W.J., Zhu, L.P., Gao, J., He, H., et al. (2015). Protective activity of salidroside against ethanol-induced gastric ulcer via the MAPK/NF-*κ*B pathway in vivo and in vitro. *IntImmunopharmacol*, 28, 604–15.

Dewi, O.S., Purwandhono, A., Sugiyanta. (2013). Pengaruh pemberian madu terhadap gambaran histopatologi lambung tikus wistar (*Rattusnovergicus*) jantan yang diinduksi metanol. (Thesis).*Artikel Ilmiah Hasil Penelitian Mahasiswa.*

Dorokhov, Y.L., Shindyapina, A.V., et al. (2015). Metabolic methanol : molecular pathways and physiological roles. *Phsiol Rev 95.*

Ibrahim, I.A.A., Abdulla, M.A., Abdelwahab, S.I., Al-Bayaty, F., Majid, N.A.(2014). Leaves extract of muntingiacalabura protects against gastric ulcer induced by ethanol in sprague-dawley rats. *ClinExpPharmacol.,* 1-6

Irramah, M., Julizar, Irawati, L. (2017). Pengaruh uncariagambirroxb terhadap ulkus gaster dan kadar malondialdehid hewan coba yang diinduksi etanol. *MajalahKedokteranAndalas*, 40, 1-10.

Jannah, A.I., Zuraida, R. (2016). Pisang (*Musa paradisiaca)* sebagai anti ulserogenik pada ulkus gaster akibat induksi obat anti inflamasi non steroid(OAINS). *MAJORITY*.;5(4):28-31

Kholifaturrokhmah, I., Purnawati, R.D. (2016). Pengaruh pemberian ekstrak buah kersen (Muntingiacalabura L) dosis bertingkat terhadap gambaran histopatologi ginjal mencit BALB/C yang hiperurisemia. *JKD,* 5(3), 199-209.

Kololu, D.F., Lintong, P.M., Loho, L. (2014). Gambaranhistopatologislambungtikuswistar (*Rattusnovergicus*) yang diberikanalkohol. *J eBM,* 2(2), 442-51

Kurniati, I.D., Rohmani, A. (2017). Ekstrak Buah Kersen (Muntingiacalabura) dalam Menurunkan Jumlah Sel Goblet pada Tikus yang Dipapar Asap RokokKersen Fruit Extract (Muntingiacalabura). *Jurnal Kedokterandan Kesehatan*, 13(2), 144–52.

Li, W., Wang, X., Zhang, H., He, Z., et al. (2016). Anti-ulcerogenic effect of cavidine against ethanol-induced acute gastric ulcer in rats and possible underlying mechanism. *IntImmunopharmacology*, 1(38), 450-59.

Manzo, A.S., Saavedra, M.A. (2010). Cellular and mitochondrial effects of alcohol consumption. *IntJ Environ Res Public Health*, 7, 4281–304.

Neurath, M.F. (2014). Cytokines in inflammatory bowel disease, Scand. *J Gastroenterol,* 14, 329–40.

Ninditya, D., Miranti, I.P., Wijayahadi, N. (2016). Pengaruh ekstrak daun kersen (*Muntingiacalabura*) terhadap gambaran mikroskopis ginjal tikus wistar jantan yang diinduksi etanol dan *soft drink*. *JKD*, 5(4), 658-664

Nugroho, A. (2012). Efektivitas seduhan daun kersen (Muntingiacalabura L.) terhadap kadar enzim endogen superoksida dismutase (SOD) padatikus diabetes mellitus yang diinduksi streptozotocin-nicotinamide (STZ-NA). *Jurnal Kedokteran Universitas Muhammadiyah,* 1-12.

Rahal, A., Kumar, A., Singh, V., Yadav, B., Tiwari, R., Chakraborty, S., Dhama, K., et al. (2014). Oxidative stress, prooxidants, and antioxidants the interplay. *BioMed Research International*, 14-9.

Rozza, A.L., Faria, F.M., Brito, A.R., Pellizzon, C.H. (2014). The gastroprotective effect of menthol: involvement of anti-apoptotic, antioxidant and anti-inflammatory activities. *J Plos One*, 9(1), e86686.

Sherwood, L. (2014). Fundamentals of human physiology. ed 4. Canada: Yolanda Cossio.

Simajuntak, K. (2012). Peran antioksidan flavonoid dalam meningkatkan kesehatan. *Bina Widya,* 23(3), 135-40.

Sulaiman, A.Y., Astuti,P., Shinta, A.D.P. (2017). Uji anti bakteri ekstrak daun kersen (*Muntingiacalabura L.)* terhadap koloni *Streptococcus viridians. Indones J Heal Sci.*, 1(2), 1-7.

Tritama, T.K. (2015). Konsumsi alkohol dan pengarunya terhadap kesehatan. *J Majorty*, 4, 7-10.

Wahyuni, R., Istiadi, H., Utami, A.W. (2017). Pengaruh ekstrak daun kersen (muntingiacalabura l) terhadap integritas mukosa esofagus tikus wistar yang diinduksi etanol dan soft drink.*JKD*, 6(2), 1156-65.

World Health Organization. (2018). The global status report on alcohol and health. Geneva Licence: CC BY-NC-SA 3.0 IGO.

Yu, C., Mei, X.T., Zheng, Y.P., Xu, D.H. (2014). Gastroprotective effect of taurine zinc solid dispersions against absolute ethanol-induced gastric lesions is mediated by enhancement of antioxidant activity and endogenous PGE2 production and attenuation of NO production. *JPharmacol*, (740), 329–36.