

## **COMPARATIVE STUDY BETWEEN THE ROLE OF THE GRAVIOLA AND ETHANOLIC EXTRACT OF *Eucalyptus camaldulensis* ON HEPATIC REGENERATION IN RABBITS MODEL**

Firas Hussain al-baw. Zainab J. Malik and A. J. AL- Khamas

Department of Pathology, college of Veterinary Medicine, Al- Qasim  
Green, Babylon, Iraq.

**Keywords:-** graviola, eucalyptus, hepatoctomy.

*Corresponding Author: firas74kadhim@yahoo.com*

### **ABSTRACT**

The aim of this study was to investigate the effect of graveola and extract of eucalyptus productions on liver regeneration. 16 mature males rabbit were used. The animals were divided into two equal groups. The animals were anesthetized by ketamine and xylazine as general anesthesia. The animals were anesthetized by atropine sulphate (1mg /Kg B.W) intramuscularly as premedication and 15 minutes later a mixture of xylazine hydrochloride (20mg /Kg B.W) and Ketamine hydrochloride (40 mg /Kg B.W) intramuscularly. After anesthetized the rabbits and preparation the site of operation was done in the upper part of abdomen, then Surgical incision was done and exposure of fourth lobe of the live. The liver handling by thump forceps and the stitches was done on the lobe. Three knots were tied and dissecting scissors were used to cut the tied lobe just distal to the suture. Then the abdominal wall was approximated with a running 3-0 Polyglactin suture, and the skin was closed with a running 2-0 polyamide suture. And after hepatoctomy divided the animals to two equal groups; first group give the graveola orally and second group give the extracted eucalyptus orally too for 15 days . The clinical parameters included temperature, respiratory , and heart rates, were within acceptable limits post operatively in all animals in two groups. The intra-abdominal adhesions in different degrees of evaluations and occurred among liver, different organs and abdominal wall that more frequency in graviola group when compared with another treated

group. Histopathological Examinations was done by taken the liver biopsies on 15<sup>th</sup> day postoperatively. The histopathological results of this study revealed that the liver regeneration similar maturity in two groups.

## INTRODUCTION

Graviola (*Annona muricata*) is a miraculous evergreen tree found in rainforest of America . This small tree is well known in herbal medicine and has several names: Soursop, Paw, Guanabana. (1). Graviola acts as an antidepressant due to the novel alkaloids found in its seeds and roots . However, one should be very careful while undergoing the treatment with this plant, because these alkaloids may be toxic to the nervous system . (2)

Eucalyptus is one of three similar genera that are commonly referred to as 'eucalypts', the others being *Corymbia* and *Angophora* . many species, though by no means all, are known as gum trees because they exude copious  kino  from any bark. (3)

## MATERIALS AND METHODS

### Materials

Equipment	Company	Origin
Centrifuge	CYAN	Belgium
Light Microscope	CYAN	Belgium
Sensitive Balance	Sartorius	Germany
Soxhlet	Atico	India
Aspirating pipettes(5ml)	Suzhou dukang medical	China
Embedding processor	Leica	USA
Eppendorf tubes	Sigma	UK
Micropipette	Eppendorf	Germany
Micotom	Leica	USA
Non- heparin tube	Suzhou dukang medical	China
Slides	Suzhou dukang medical	China
Surgical sate	Suzhou dukang medical	China
Syringe	Suzhou dukang medical	China
Test tubes	Suzhou dukang medical	China
Tissue processor	Leica	USA
Water bath	Kottermann	Germany
Suture Materials		

## Methods

Preparation of phosphate buffered saline (PBS) at PH 8

That prepared by dissolving the following chemicals in 1 L of distilled water

### Substance:-

Sodium chloride 8gm

Potassium chloride 0.2gm

Potassium dihydrogen phosphate 0.2gm

Sodium dihydrogen phosphate 1.15gm

Distilled water 100ml

### Plant leaves collection:-

The Eucalyptus leaves were collected during the period that extended from the park of veterinary medicine college of Al- Qassim Green University , so we washed the leaves to get rid of dusts and then dried inside the hot air oven (100° C) and then grinded to fine powder and saved in plastic bags . At the end stored inside the refrigerator until use. Preparation of the ethanolic extract (*eucalyptus camaldulensis*):- twenty gms were taken from powdered leaves and extracted with ethanol 70% by soxhlet apparatus for 24hr. and then the extract was taken and placed in a petri dish inside the oven at 40° C for 48hr. and stored at 4°C until used (4)

## Experimental design

16 adult male rabbits at age (10- 12) months, and weighted between ( 1.5- 3) kg. they were adapted at the animals house for 2 weeks before starting the experiment.

They divided to two groups and all groups had partial hepatoctomy from the third lobe

1. The first group (8 animals):

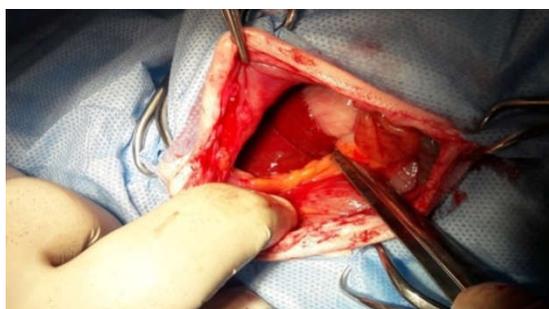
After doing partial hepatoctomy give the animals orally the Graviola for 15 days

2. The second group (8 animals):

After doing partial hepatectomy give the animals orally the ethanol extract of eucalyptus camaldulensis leaves (200mg/ kg b.w.) for 15 days

### **Surgical procedure**

**Surgical Procedures:** Hepatic resection was performed as described previously by (5). The rabbits were anesthetized by 0.8 mg/kg intramuscular Acepromazine, 0.05 mg/kg Buprenorphine and 40 mg/kg of Ketamine then a midline laparotomy was performed. Surgical incision was done in the upper part of abdomen, and exposure of third lobe of the liver (Fig. 1), The liver handling by thump forceps (Fig. 2), then the lobe partial resected gently (2\*1.5 cm) with a silk 3-0 suture tie was placed underneath it and positioned as proximal to the origin of the lobe as possible. The two ends of the suture were tied over the lobe of liver at its base near the inferior vena cava (Fig.3). Three knots were tied and dissecting scissors were used to cut the tied lobe just distal to the suture. Then the abdominal wall was reapproximated with a running 3-0 Polyglactin suture, and the skin was closed with a running 2-0 polyamide suture. (6and 7).



**Figure (1): The midline laparotomy was performed. Surgical incision was done in the upper part of abdomen and exposure of fourth lobe of the live.**



**Figure (2): The exposure of third lobe of the liver and handling by thump forceps**



**Figure (3): The two ends of the suture were tied over the top of the lobe at its base near the inferior vena cava then surgical removed done.**

## **RESULTS AND DISCUSSION**

### **Clinical examination**

The results of the physical and clinical examination for temperature, respiratory rate, heart rate, defecation and urination during first week post operation revealed that, slight elevated in temperature, respiratory rate and heart rate with seen normal defecation and urination in all animals. Significant convergence in results between the control group since the second days postoperation and treated group but early disappeared in treated group when compared with control group at 3<sup>rd</sup> and 5<sup>th</sup> days postoperation respectively, that may be due to increase the blood flow in operative area. Beside that increase dilatation of blood vessels with increase permeability of capillaries was agreed with other workers (8) whom mentioned that there were no significant changes recorded in these clinical parameters before and after surgical operation.

The animals activity which was represented by animal posture, motion, and alert to the surrounding) and also the appetite in present study, were not altered. However there were four animals in control group have normal appetite but limited activity during three days postoperatively, this is may be attributed to visceral pain or adhesion formation. The visceral pain during the first hours after surgical intervention or pain associated with adhesion formation postoperatively reported in previous researches (9,10).

**Adhesions:**

The intra-abdominal adhesions more frequency in treated group by graviola than in treated group by eucalyptus camaldulensis leaves in different degrees of evaluations and occurred among liver, different organs and abdominal wall. Quantitative evaluation (MSAS) (Table-1-) showed that graviola group had large number (3 animals) of adhesion at grade 2, while the treated group by eucalyptus

**Table -1- Multiple sites adhesions schemes (MSAS).**

Table 1 Groups	Number of animals for each grade				
	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Graviola group	1	3	3	1	0
eucalyptus camaldulensis	5	1	2	0	0

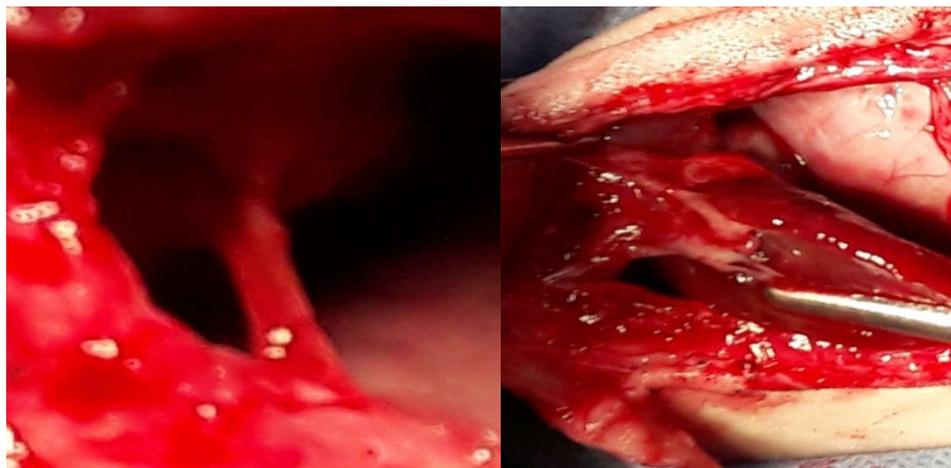
camaldulensis had large number (5 animals) of adhesions at grade 0, (1 animals) at grade1 and (2animals) at grade 2 therefore the entrance to the abdominal cavity and easy of visualization to the abdominal organs.

Qualitative evaluation (ATTS) (Table-2-) showed that graviola group had grade2 adhesions at (5 animals). While treated group had grade 0 adhesions at (8 animals). Therefore the adhesions in treated group had little effect and easy to perform adhesiolysis comparing with control group Adhesions formation is part of normal healing process (Betsabee *et al.*, 2017).

One of the most potent stimuli for initiation of an inflammatory response and thus adhesions formation is surgical trauma. Routine surgical procedures involve various degree of tissue handling that initials tissue abrasion, desiccation, ischemia, bleeding, infection and exposure to foreign materials, any of these factors can initiate inflammatory responses which eventually lead to adhesion formation (Liakakos *et al.*, 2001).

**Table-2- Adhesive tissue tenacity schemes ( ATTS ).**

Table 2 Groups	Number of animals for each grade				
	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Graviola group	2	2	4	0	0
eucalyptus camaldulensis	3	2	1	0	0



**Figure (4)** Macroscopic view shows grade 2 adhesion (MSAS) among liver, omentum and abdominal wall in graviola group (A) and **eucalyptus camaldulensis** group(B).

#### **Hsitopathological Findings:**

The histopathological findings of the liver after partial hepatectomy were as following:

The histopathological examination in treated group by Graveola there was early epithelization and dilation the central vein

But the histopathological examination in treated group by extract of eucalyptus camaldulensis leaves show the inflammatory cells with granulation area but in another section show dilation the bile duct

When we were showing the histopathological results, we had a view the graveola resulting a good healing in liver through 15<sup>th</sup> days this due to many properties of it, so due to novel alkaloid found in it is seeds and roots it is act an antidepresent (2). As well as; have beneficial elements including antiviral , anti-parasite and anti-inflammatory properties (2).

But in the histopathological results of extract of eucalyptus camaldulensis we were show have a good healind too but lesser than the group of graveola in rather , so it was have anti- inflammatory properties and have capable to inhibitor of cytokines and stimulated the phagocytosis ( 13 and 14) and have lipopolysaccharide in nature that very good for healing (15)

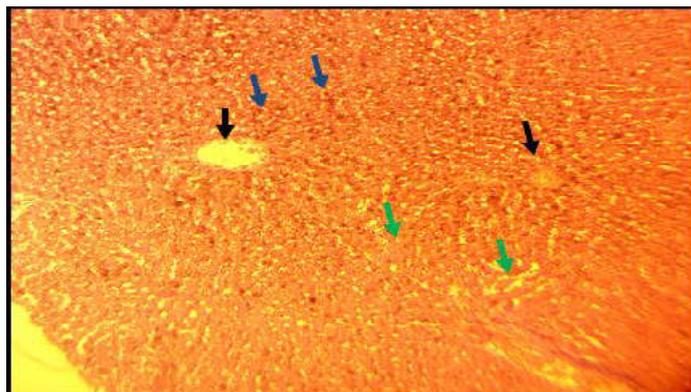


Fig.1: Histological section in the liver of SKL group showed dilation with congested central vein (black arrows), dilated sinusoids (green arrows) and inflammatory cells infiltration (blue arrows). H&E stain. 10X.

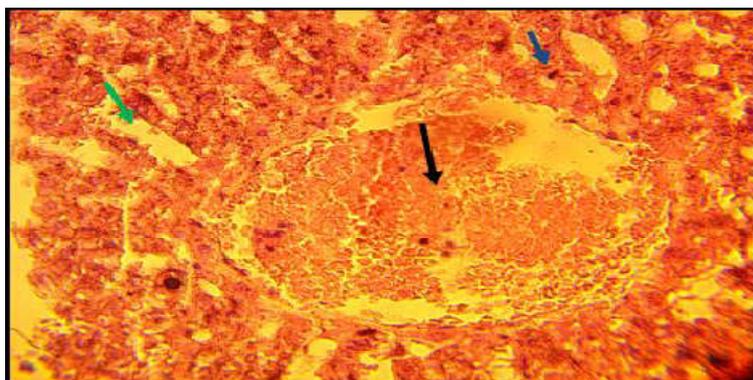


Fig.2: Histological section in the liver of SKL group showed dilation with congested central vein (black arrow), dilated sinusoids (green arrow) and inflammatory cells infiltration (blue arrow). H&E stain. 40X.

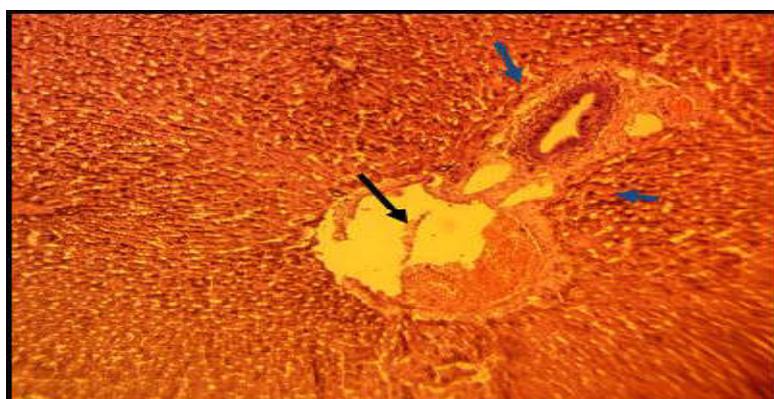


Fig.3: Histological section in the liver of SKL group showed dilation with congested hepatic artery (black arrow) and inflammatory cells infiltration around bile duct (blue arrow). H&E stain. 10X.

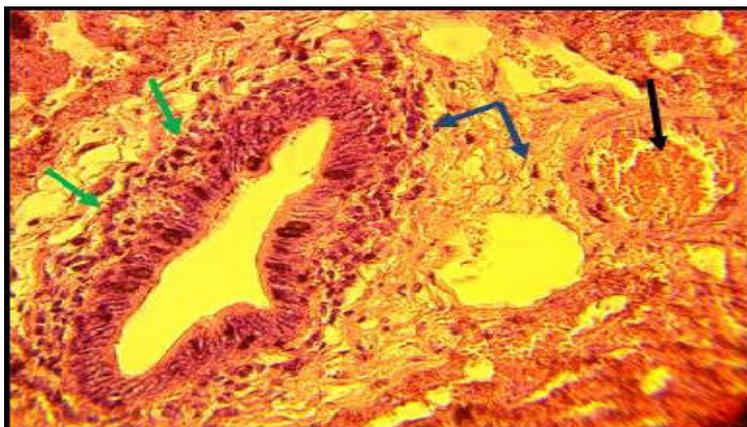


Fig.4: Histological section in the liver of SKL group showed dilation with congested hepatic artery (black arrow) , inflammatory cells infiltration (blue arrows) and dilation of bile duct as well surrounded by inflammatory cells (green arrows). H&E stain. 40X.

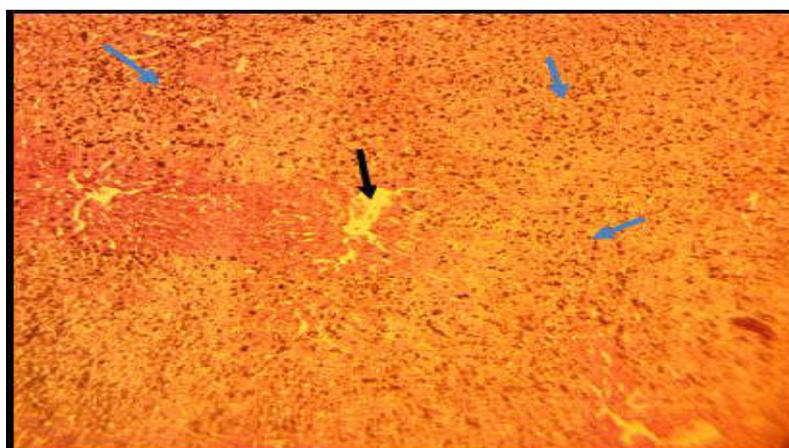


Fig.5: Histological section in the liver of SQR group showed dilation of central vein (black arrow) and severe inflammatory cells infiltration in entire hepatic parenchyma (blue arrows). H&E stain. 10X.

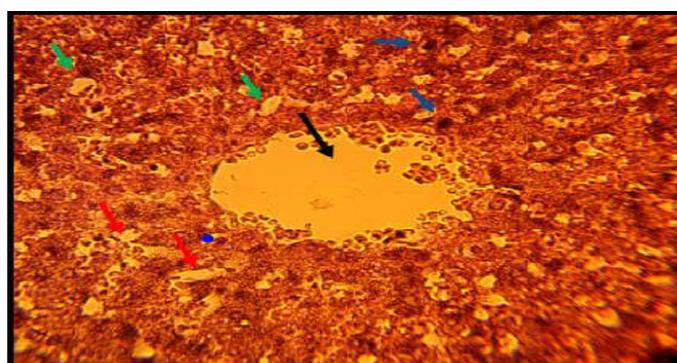


Fig.6: Histological section in the liver of SQR group showed dilation of central vein (black arrow) , inflammatory cells infiltration (blue arrows) and vacuolation of hepatocytes (green arrows) as well as dilation of sinusoids (red arrows). H&E stain. 40X.

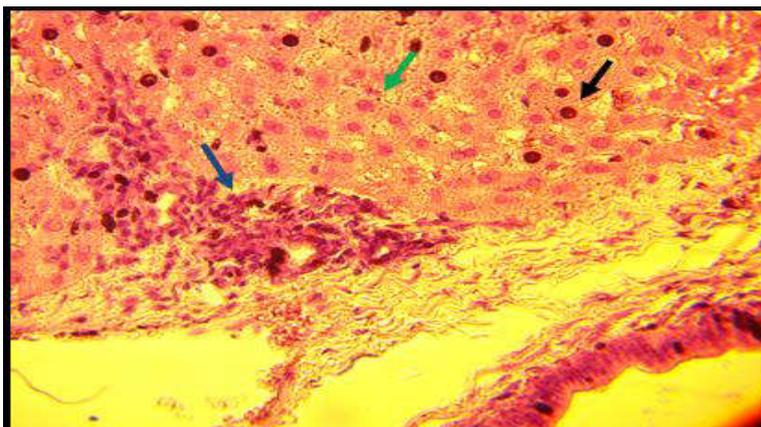


Fig.7: Histological section in the liver of SQR group showed inflammatory cells infiltration (blue arrow) and vacuolation of some hepatocytes (green arrows) and pyknotic hepatocytes (black arrow). H&E stain. 40X.

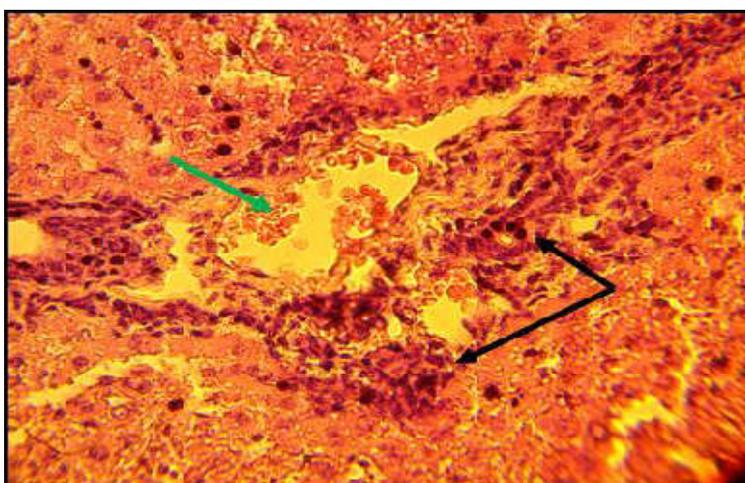


Fig.8: Histological section in the liver of SQR group showed inflammatory cells infiltration (black arrow) and congestion of portal vein (green arrows). H&E stain. 40X.

## REFERENCES

1. Chang, F.R. (2003).New adjacent bis- tetrahydrofuran annonaceous acetogenins from *Anon muricata*. *Planta Med.* 69(3): 241- 6.
2. Rottscholl, R.; Haegele, M. and Jainsch, B. (2016). Chronic consumption of *Annona muricata* juice triggers and aggravates cerebral tau phosphorylation in wild- type and MAPT transgenic mice. *Journal of Neurochemistry.* 139(4): 624- 639.
3. Gledhill, D. (2008). *The Names of Plants* (4ed). Cambirdge University Press. P. 158.

4. Harborne J. B. (1999). Phytochemical dictionary: Handbook of bioactive compounds from plants 2<sup>nd</sup> ed.
5. Higgins, G.M. and R.M. Anderson, 2002 Experimental pathology of the liver: Restoration of the liver of the white rat following partial surgical removal. *Archives of Pathology*, 9: 188.
6. Foster, R., M. Zimmerman and J.F. Trotter, 2007. Expanding the donor options: marginal, living and split donors. *Clinics in Liver Disease*, 11: 417-429.
7. Yacine, K. A., Karim, A., Houari, H., Mokhtar, B., & Mohamed, M. (2014). Histological Study of Liver Regeneration Following Partial Hepatectomy and Total Splenectomy. *World Journal of Medical Sciences*, 11(4), 468-472.
8. Verma, G. R.; Lyngdoh, T. S. and Kaman, L. (2006). Placement of 0.5% bupivacaine-soaked surgical in the gallbladder bed is effective for pain after laparoscopic cholecystectomy. *Surg. Endosc.*, 20:1560-1564.
9. Nakajima, J.; Sasaki, A.; Toru-obuchi, T.; Baba, S.; Nittaand, H. and Wakabayashi, C. (2009). Laparoscopic Subtotal Cholecystectomy for severe Cholecystitis. *Surg. Today.*, 39(10):870-875.
10. Lu, Y., Liu, P., Fu, P., Chen, Y., Nan, D., & Yang, X. (2017). Comparison of the DWI and Gd-EOB-DTPA-enhanced MRI on assessing the hepatic ischemia and reperfusion injury after partial hepatectomy. *Biomedicine & Pharmacotherapy*, 86, 118-126.
11. Betsabee, O. S. I., Luis, S. S. J., Arturo, R. S. J., & Montserrat, C. S. (2017). Evaluation of the toxicity and pathogenicity of biocontrol agents in murine models, chicken embryos and dermal irritation in rabbits. *Toxicology Research*.
12. Laikakos, T.; Thornakos, N.; Fine, P. M. et. al., (2001). Peritoneal adhesions: Etiology, pathophysiology, and clinical significance. Recent advances in prevention and management. *Dig. Surg.*, 18:260-273.
13. Nagpal, N.G. Shah, M. Arora N., et al (2010). Phytochemical and Pharmacological Aspects Of Eucalyptus Genus, *International Journal Of Pharmaceutical Sciences And Research*, 1(12), 28-36.
14. Serafino, A.; Sinibaldi, P. and Andreala, F. (2008). Stimulatory effect of eucalyptus essential oil on innate cell- mediated immune response. *BMC*.

15. Zhou, J.Y.; Wang, X.F. and Tang, F.D. (2007). Inhibitory effect of 1,8-cineol on Egr-1 expression in lipopolysaccharide-stimulated THP-1 cells. *Acta Pharmacol Sin.* 28: 908- 912.