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Emerging and Re-emerging Animal Health Challenges in Low and Middle-Income Countries

Experimental Evaluation for Graviola Extracts and Eucalyptus Extract's Capacity to Induce Healing of Hepatocytes in Rabbits Model

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Abstract | The objective of studying this research is to discuss the influence of Graviola and eucalyptus extract and their relationship to liver cell regeneration. We used sixteen mature male rabbits by dividing the animals into two groups. As a general anesthesia by using xylazine ketamine and rabbits were anesthetized by using atropine sulfate (1 mg/kg B.W.) inter-muscularly than after 15 min rabbits were anesthetized intramuscular with a mixture consisting of ketamine hydrochloride (40 mg/kg of B.W. and xylazine hydrochloride (20 mg/kg B.W.). After anesthetizing the animals, in the upper part of the abdomen the location of operation is determine, and the incision surgically made exposing the fourth lobe of the animal. The liver was manipulated with impact forceps, and stitches were applied to the lobe then by using the surgical scissors to cutting the tied lobe away from the suture after applied the three knots were tied, with a 3-0 polyglactin suture the wall of abdominal then re-approximated, and by used a 2-0 polyamide suture the skin was closed. After liver resection, the animals were divide into two equivalent groups; Graviola was given orally to the first group, and eucalyptus extract was given orally for 15 days to the second group. The animals in the both experimental groups that the parameters clinically were including temperature, rate of respiration and heart rate were with in agreeable values postoperatively. However, adhesions of intra-abdomen wall between the liver, various organs, and the wall of abdomen occurred in a varying degree, which were more frequent in the graviola group than in the other treated group. On the 15th day postoperatively histological examinations were performed by using the liver biopsy method. The pathological anatomical outcomes of this study presented that the maturation in the second group and the regeneration in liver were similar. In the graviola group when compared to another treated group the adhesions of the intra-abdominal were in various degrees of valuation and manifestation between the liver, different organs and the wall of abdomen more than frequently. On 15th day postoperatively taking the liver biopsies for histopathological examinations. Histopathologically the results of this research reveal that the liver regenerate is comparable to maturity in the second group.

Keywords | Graviola, Eucalyptus, Hepatoctomy, Rabbits**Received** | July 05, 2024; **Accepted** | October 09, 2024; **Published** | December 05, 2024***Correspondence** | Zainab J. Malik, Department of Pathology and Poultry Diseases, Collage of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq; **Email:** Zainab1987@vet.uoqasim.edu.iq**Citation** | Shaheed HS, Malik ZJ, Alneamah GAA, Salih TH (2024). Experimental evaluation for graviola extracts and eucalyptus extract's capacity to induce healing of hepatocytes in rabbits model. J. Anim. Health Prod. 12(s1): 213-217.**DOI** | <https://dx.doi.org/10.17582/journal.jahp/2024/12.s1.213.217>**ISSN (Online)** | 2308-2801**Copyright:** 2024 by the authors. Licensee ResearchersLinks Ltd, England, UK.This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

INTRODUCTION

The Graviola (*Annona muricata*) is one of the wonderful evergreen trees found in New York City, America. This

small tree is considered one of the most common trees in liberal herbal medicine and has different names such as: Soursop, Paw, Guanabana (Chang, 2003). Graviola, as an antidepressant, is due to the new class of alkaloids found

in it in stores and seeds. However, it must be very careful during treatment, because of those alkaloids may be toxic to the nervous system (Rottscholl *et al.*, 2016).

The Eucalyptus tree is classified as an extension of one of the three genera, which is known as Eucalyptus, while the other genera are called Corymbia and Angophora. Which includes many species and many species and is widespread, although it cannot be determined definitively in any case due to the fact that it is the strict decision maker sorting out the prolific Keno from any coincidence (Gledhill, 2008).

MATERIALS AND METHODS

SAMPLE COLLECTION

The Eucalyptus leaf specimen was obtain commencing from the park of the College of Veterinary Medicine at Al-Qasim Green University, and to get rid d of dusts the leaves was washed and then dried in the hot air oven at (100°C) and then pulverize into fine powder and set aside in the plastic bags. Latterly, stored in the refrigerator until use. 20 gm were in use from the leaves powder and extract with using 70% of ethanol through soxhlet apparatus for 24 hours to preparation the ethanolic extract of (*Eucalyptus camaldulensis*) then the extract was possessed and culture in a petri-dish and put inside the oven at 40°C for 48 hours and store at 4°C until it's use (Harborne, 1999).

DESIGN OF EXPERIMENTAL

The experimental applied by using sixteen adult male rabbits at average age were (10-12) months, and there weight were between (1.5-3) kg. and at the animal house the rabbits were adopted for two weeks before starting the experiment. The experimental groups spilt into two groups , and the partial hepatoctomy from the third lobe of liver had done to all groups as following:

1. The first group of experimental were (8 animals): The Graviola give orally for 15 days after doing partial hepatoctomy.
2. The second group of experimental were (8 animals): The ethanolic extract of Eucalyptus camaldulensis leaves (200 mg/kg b.w.) give orally for 15 days after doing partial hepatoctomy.

SURGICAL PROCEDURE

As described previously the hepatic resection was done according to (Higgins and Anderson, 2002). By using 0.8 mg/kg intra-muscular acepromazine, 0.05 mg/kg buprenorphine and 40 mg/kg ketamine the rabbits were anesthetized and then performed a midline laparotomy. In the upper abdomen, a surgical incision was made and the third lobe of the liver was exposed (Figure 1) by using thump forceps the liver was handling (Figure 2), Using a 3-0 silk suture, the partial lobe is gently amputated (2*1.5

cm) and as possible the origin of the lobe of liver placed underneath it and positioned proximally, At the base of the liver and near the inferior vena cava, sutures were tied over the liver lobe (Figure 3) by using surgical scissors to cut the tied lobe away from the suture, three knots were tied and then the abdominal wall was re-approximated with a management by using 3-0 Polyglactin suture, By using surgical scissors to cut the tied lobe away from the distal part after tying three knots with a management by using 3-0 Polyglactin suture the abdominal wall was re-approximated, and with a management by using 2-0 polyamide suture the skin was closed (Foster *et al.*, 2007; Yacine *et al.*, 2014).

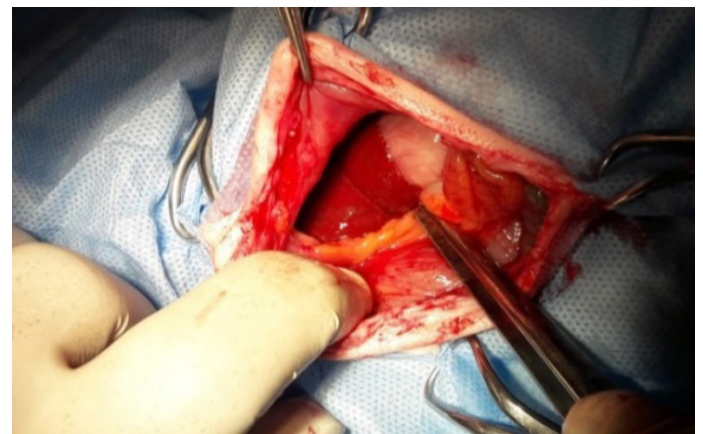


Figure 1: Gross section showed a midline laparotomy. In the upper abdomen, exposing the fourth lobe of the liver, a surgical incision was made to perform a partial liver resection in rabbits.

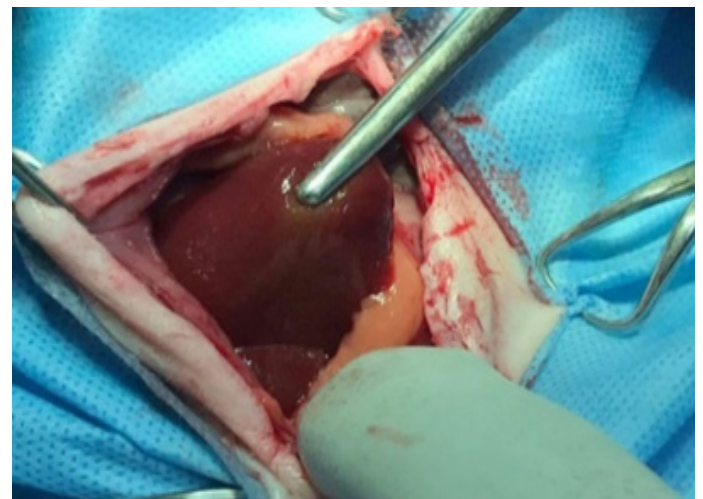


Figure 2: Gross section showed handling by use thump forceps and revelation of the third lobe of liver a for doing partial hepatoctomy in rabbit.

RESULTS AND DISCUSSION

CLINICAL EXAMINATION

During first week post operation the clinical and physical examination results showed: Heart rate, respiratory rate,

temperature, urination, and defecation revealed that minor increased in heart rate, rate of respiration and temperature, with seen normal urination and defecation overall experimental animals. Since the second days post-operation there is Significant assemblage in the results between the control group and treated group but respectively post-operation there is early disappeared in treated group when compared with control group at 3rd and 5th days, that may be due to increase in the blood flow to the operative area. In addition the blood vessels dilatation increase with increase in the permeability of capillaries was agreed with other researcher (Verma *et al.*, 2006), whom mentioned that the clinical parameters before and after surgical operation were no significantly changes recorded.

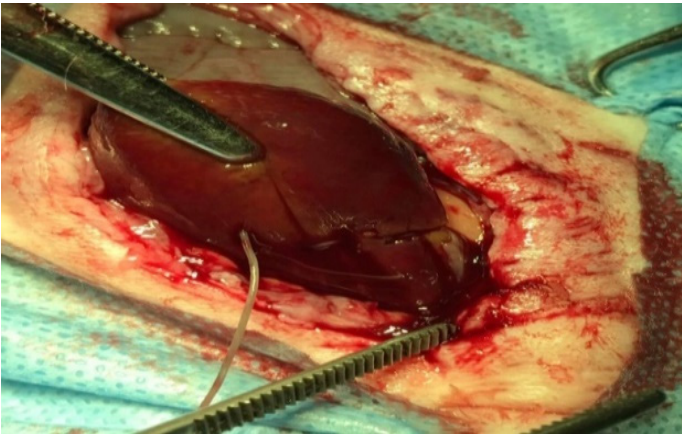


Figure 3: Gross section showed the base of the liver and near the inferior vena cava, sutures were tied over the liver lobe and surgical removed done for doing partial hepatectomy in rabbit.

The animal performance which was represent by posture of animal, alert to the surrounding and motion) with observing that the appetite were not altered in present study. In the control group there were limited movement during three days postoperatively and but about four animals have a normal appetite, this is maybe refers to visceral pain or adhesion formation. postoperatively Adhesion formation reported in previous researches as well as the visceral pain during the first hours after surgical intervention or pain is recorded (Nakajima *et al.*, 2009; Lu *et al.*, 2017).

Adhesions in graviola treated group the adhesions in the intra-abdomen was more than frequent in Eucalyptus camaldulensis leaves treated group assessments and occurred between liver, different organs and abdominal wall were in variable degrees, evaluation of quantitative (MSAS) (Table 1) showed that the graviola group had a high number (3 animals) of adhesions at grade II, while the group treated with Eucalyptus camaldulensis had a high number (5 animals) of adhesions at grade 0, (1 animal) in the first row and (2 animals) in the second row, thus accessing the abdominal cavity and making it

easy to clearly see the abdominal organs. The qualitative evaluation (ATTS) (Table 2) showed that the graviola group had grade II adhesions in (5 animals). While treated group had grade 0 adhesions at (8 animals). Therefore, the release of adhesions was easy to perform and the adhesions in the treated group had little effect compared to the control group and showed that the formation of adhesions is part of the natural healing process (Betsabee *et al.*, 2017). Surgical trauma is considered one of the strongest triggers for initiating the inflammatory response that leads to the formation of adhesions. There are several factors, some of which include routine surgical procedures, which include varying degrees of tissue handling that lead to tissue erosion, bleeding, ischemia, dehydration, infection, and exposure to foreign materials. Any of these factors can lead to inflammatory responses that lead to end to adhesion formation (Laikakos *et al.*, 2001).

Table 1: Multiple sites adhesions schemes (MSAS).

Animals number of each grade					Groups
Grade 4	Grade 3	Grade 2	Grade 1	Grade 0	
0	1	3	3	1	First group
0	0	2	1	5	Second group

Table 2: Adhesive tissue tenacity schemes (ATTS).

Animals number of each grade					Groups
Grade 4	Grade 3	Grade 2	Grade 1	Grade 0	
0	0	4	2	2	First group
0	0	1	2	3	Second group

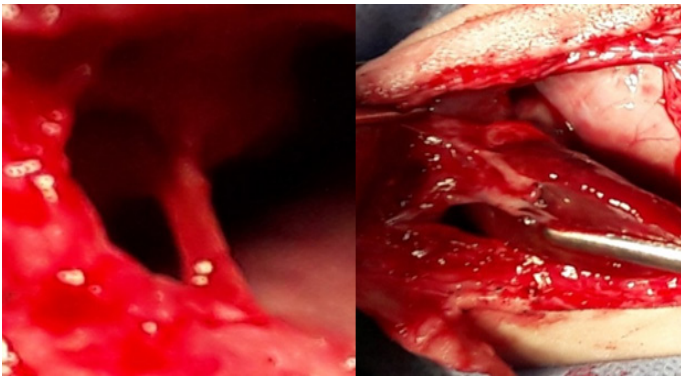


Figure 4: Gross section showed grade II adhesion (MSAS) between the liver, omentum, and abdominal wall in the Graviola group (A) and Eucalyptus camaldulensis group (B).

HISTOPATHOLOGICAL RUSTLES

After partial hepatectomy liver histopathologically showed as follow: in treated group by Graviola the histopathological examination revealed there was dilation in the central vein with early epithelization, While in extract of Eucalyptus camaldulensis leaves treated group the histopathological examination showed granulation area with inflammatory

cells present when compared with other section showed the bile duct is dilated, the histopathological results revealed that the graviola ensuing through 15th days liver had a good healing because of the new alkaloid it contains in the seeds and roots and because of its many properties it can be an antidepressant (2). As well as, have useful elements containing anti-inflammatory properties, anti-parasite and antiviral (2). In the second group of extracts of *Eucalyptus* treated the liver showed histopathologically magnification of regeneration and healing but lower than occurs in first group treated with Graviola (Nagpal *et al.*, 2010; Serafino *et al.*, 2008; Zhou *et al.*, 2007).

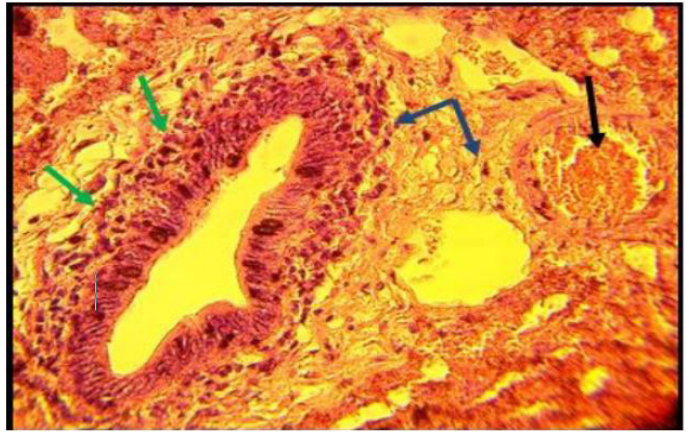


Figure 7: Histopathological section of a rabbit liver from *Eucalyptus camaldulensis* leaf group extract showed mild regeneration of hepatocyte of liver (arrow) (staining with H and E stain 10X).

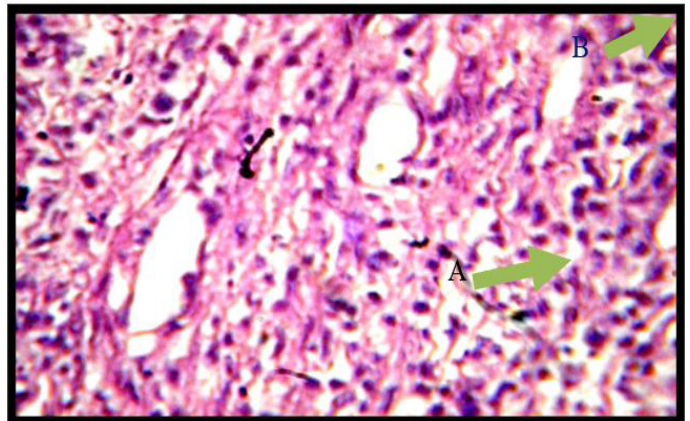


Figure 8: Histopathological section of liver related to treated group to extract of *eucalyptus camaldulensis* leaves, shows infiltrated mononuclear cells (A) and appear of myofibroblast (B), with granulation tissue formation (staining with H and E stain 10X.).

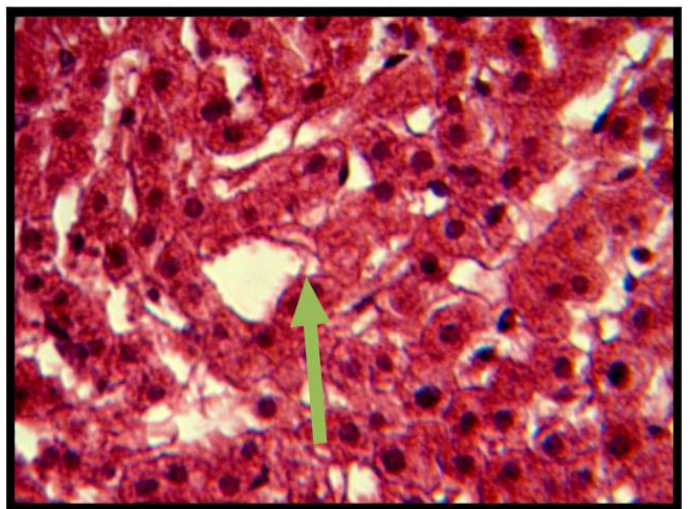


Figure 9: Histopathological section The liver after returning to its normal structure, which is represented by hepatocytes cord around the central vein (arrow) (staining with H and E stain 100X).

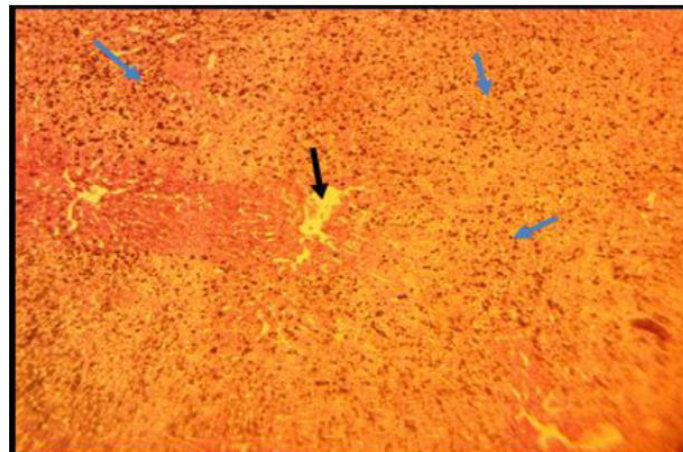


Figure 5: Histopathological section of liver of rabbit of Graviola group showed heavy inflammatory reaction infiltration in entire hepatic parenchyma (blue arrows) with dilation of central veins (black arrow) (staining with H and E stain 10X).

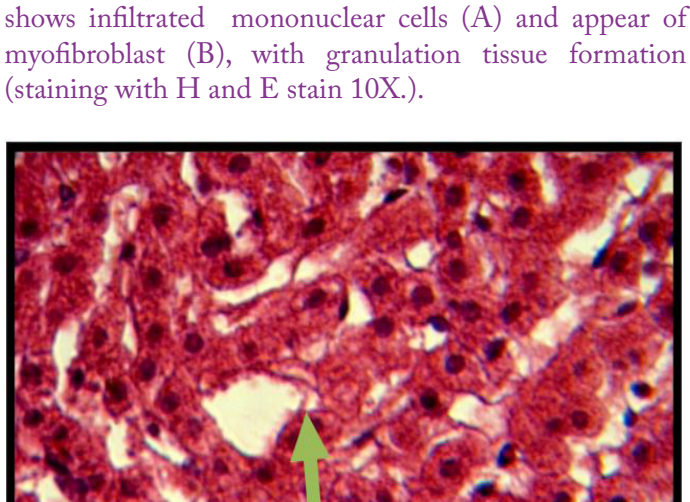


Figure 6: Histopathological section of liver of rabbit of *eucalyptus camaldulensis* extract of leaves group showed infiltration of inflammatory cells (blue arrows) and the bile duct is dilated and surrounded by inflammatory cells (green arrows) in addition to the dilation there is congested of hepatic artery (black arrow), (staining with H and E stain 40X.)

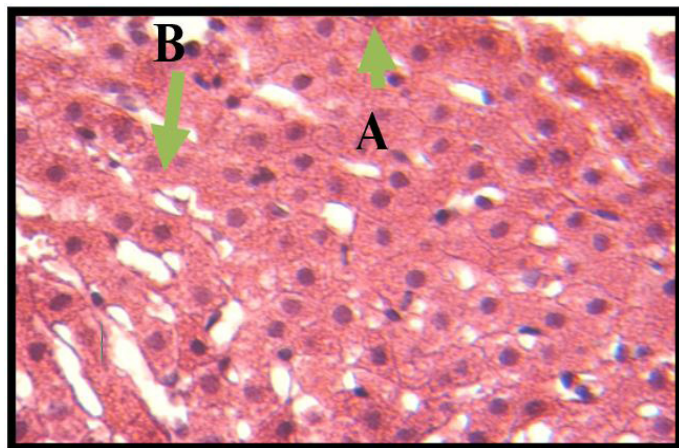


Figure 10: Histopathological section of liver related to Graviola treated group, shows normal hepatic architecture which represented by normal lobules, CV (staining with H and E stain 10X).

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NOVELTY STATEMENT

This study influence of Graviola and eucalyptus extract and their relationship to liver cell regeneration.

AUTHOR'S CONTRIBUTION

All authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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