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EFFECT OF COMMIPHORA MYRRHA ON SCIATIC NERVE REGENERATION

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ABSTRACT

Commiphora myrrha, species of the Burseraceae family, Seven sesquiterpenes, named commiterpenes A−C and myrrhterpenoids K−N (1−4), have been isolated from the resin of Commiphora myrrha showed moderate neuroprotective effects. In this study, 15 New Zealand 35-36 week old and weighing 2.5-3 kg male rabbits were used, and randomly divided into four groups (A, B, and C) of 5 rabbits each, according to the procedure performed. Our findings suggest that rabbit underwent Commiphora myrrha repair had fewer postoperative complications, muscular and vascular injuries and appeared to have less discomfort and to return to their usual activities more rapidly.

Keywords: Commiphora myrrha, Sciatic Nerve, Rabbit

INTRODUCTION

Commiphora myrrha, species of the Burseraceae family, are native East African species, Africa and most of the Middle East, India, found in Kenya, Uganda, Tanzania, Ethiopia and Somalia comprises more than 150 species that produce fragrant resins commonly known as myrrh,**[1-4]**. *Commiphora* species are small trees or shrubs with short, thorny branches which has hairless toothed leaves with a large terminal leaflet and two tiny lateral leaflets. Myrrh is the exudates produced by secretory tissue in the bark of Commiphora species. True myrrh is produced by *C.* myrrha, however **[5, 6]**. Commiphora myrrha is locally known as Morr Hegazi or Myrrh and Commercially as Arabian Myrrh or Karam **[7]**. The myrrh's Gum-resin-volatile oil are the main used parts, where it contains (30-60%) gum including acidic polysaccharides, resin(25-40%), volatile oil (3-8%), heerabolene, eugenol and many furansesquiterpenes **[5, 8, 9]**. Commiphora species, are important products used in pharmaceutical industries the food industry, flavours, liqueurs and Beverage, cosmetics and perfumery, and in the traditional medicines of several countries. Frankincense and myrrh are also widely used in unprocessed form for fragrances in many religious rituals, in Orthodox and Roman Catholic Churches as well as in the Muslim religion **[10]**. Commiphora has been used for treatment of fever, stomach complaints, antitumour activity, heal wounds, diseases of gall bladder, skin infections, amenorrhea, ache, dysmenorrhea, tumors, chest ailments, pain, arthritis, fractures, obesity, para- sitic infection and gastrointestinal diseases, and snake bites **[11, 12]**. Also it is an excellent remedy for mouth and throat problems, with a drying, slightly bitter taste, atherosclerosis, hemorrhoid, heptoses, high cholesterol, stomatosis, immunodepression and hyperglycemia **[8]**. More recently, neuroprotective effects of Commiphora myrrha has been studied. Seven sesquiterpenes, named commiterpenes A−C and myrrhterpenoids K−N (1−4), have been isolated from the resin of Commiphora myrrha showed moderate neuroprotective effects **[2, 4]**.

MATERIALS AND METHODS

In this study, 15 New Zealand 35-36 week old and weighing 2.5-3 kg male rabbits were used, and randomly divided into four groups (A, B, and C) of 5 rabbits each, according to the procedure performed. All rabbits were kept in individual cage during the whole experimental period, under strict hygienic conditions and fed with standard ration for rabbits and water ad labium. The animal was then anesthetized with intramuscular ketamine hydrochloride (Alfasan; 35 mg/kg) and xylazine (Rompun; 5 mg/kg). Hind limb was shaved, scrubbed with providone-iodine and alcohol, frequently. The site was scrubbed alternately with each solution three times to allow 5 minutes of contact time, and then draped in a sterile fashion. Dorsolateral approach to the rabbit sciatic nerve was done.

Blunt separation of the septum between the semitendinosus muscle and the caput pelvinumbicipitisfemoris muscle, allows for excellent access to sciatic nerve. In all groups the nerve was sharply cut with scalpel while supported with nerve holding forceps to minimize nerve stump injury. Then the nerve repair was performed as follows: group A: epineural repair with only three sutures; group B: epineural repair with three sutures and eucerin, and group C: epineural repair with three sutures and Commiphora myrrha 2.5%. The nerve coaptations were achieved using 8–0 monofilament nylon with swaged –on, fine, taper-point microsurgical needles. The nerve was covered with tensor facial lata for preserving extractions on nerves. The surgical sites were closed in layers, and the animals were allowed unrestricted movement directly after the anesthesia. All rabbits during the postoperative period (3 days after surgery), antibiotics and analgesic therapy were continued and were closely monitored for inflammation and infection. The tissue samples were fixed in 10% buffered formaldehyde solution (in phosphate-buffered saline), dehydrated in graded ethanol, embedded in paraffin (Tissue-processor, Leica, Jung histokinette 2000, Germany), sectioned at 6 micrometers (Sliding microtome, Leica, Jung histocuts, Germany) and stained with hematoxylin and eosin (H&E) for structural characteristics and Masson's trichrome for demonstration of collagen in tissue sections (Luna, 1968). Histopathologically, the sections were examined under light microscopy (Olympus, CH36 RF200, Japan) and digitally photographed with a photomicrograph (Olympus DP12, U-TVO.5XC-2, Japan).

Statistics

Quantitative results evaluated and statistical analysis carried out. First of all, data were statistically analyzed by normality tests of Kolmogorov-Smirnov and Shapiro-Wilk. The results indicate that data was not following Gaussian distribution. Therefore, the statistical analysis performed using Kruskal-Wallis test and Dunn's Multiple Comparison Post-Test. Data which obtain from triplicate samples (n=3), expressed as mean standard deviation (SD) and the value of $P<0.05$ was considered statistically significant.

RESULT

Macroscopic Observation

In this study, all the animals were survived throughout the 45 days experiment. Necropsy of the animals revealed no abnormalities or serious post-surgical complications in visceral organs and the surgical region. Macroscopically, there was no neoplasia, extensive infection or impaired wound healing in any of the rabbits of the groups.

Most of the animals in group A and B (control and placebo) presented extensive fibroplasia. In these groups, the severity of adhesions was more than for other group. As expected, most of the animals treated with 2.5% Comniphora myrrha (group C) presented little adhesion and fibroplasia. In this group, the fibroplasia was considerably less than for other groups ($P<0.05$). Comniphora myrrha reduced level of the adhesion and fibroplasia of sciatic nerve to peripheral tissues.

Histopathological Findings

In present study, the histopathologic examination of sections of all groups revealed dense fibrous connective tissue in the wound site that resulted in well formation of scar tissue. In group A and B, repair took long time and resulted in considerable fibrous scaring containing many collagen fibers and active fibroblasts and fibrocyte-type cells. The defect was completely filled with connective tissue containing thin and relatively parallel collagen fibers.

In group C the defect was filled by collagen fibers bundles dispersed in a plane parallel to the longitudinal axis of the sciatic nerve with small foci of granulation tissue. Fibroblasts and collagenous fibers mainly situated parallel and collagen accumulation was better when compared with group A and B. In this group, the cells diminished in number in compare with group A and B. Results of evaluation of amount of collagen synthesis, number of Schwann cells, number and diameter of myelinated fibers and blood vessels are presented in. Most of the animals in group A and B (control and placebo) presented moderate to severe degree of fibroplasia. In this group, the ability of nerve regeneration reaction was less than for group C. Most of the animals treated with Comniphora myrrha (group C) presented more nerve fibers regeneration and little inflammation. Comniphora myrrha. In this group, the severity of fibroplasia was significantly less than for group A and B ($P<0.05$). These findings indicated proper wound healing with Comniphora myrrha in repair of the sciatic nerve and relatively in group C. (2.5% Comniphora myrrha).

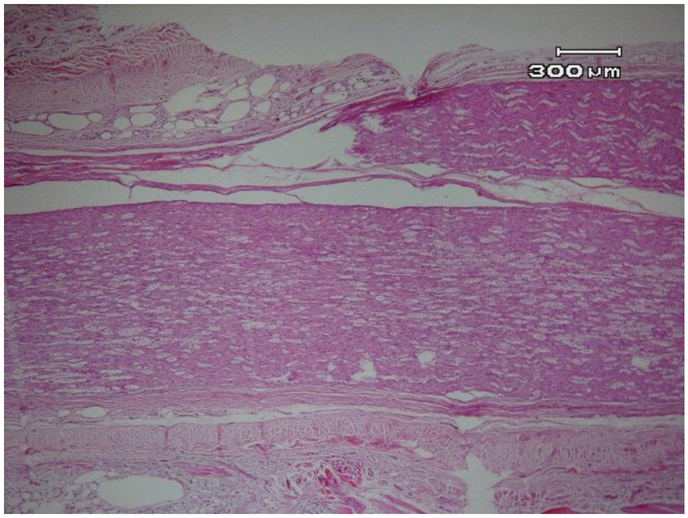


Figure 1: Regeneration of the Sciatic Nerve is Clear in Group c (Commiphora myrrha 2.5%) (H & E, 40)

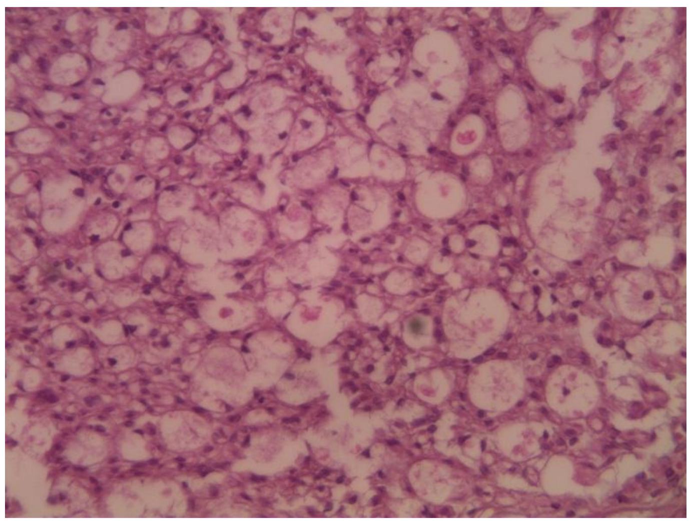


Figure 2: Section of the Sciatic Nerve. It Shows Inflammation of Axons and Vacuolization of Nerve Fibers (H&E, 400X)

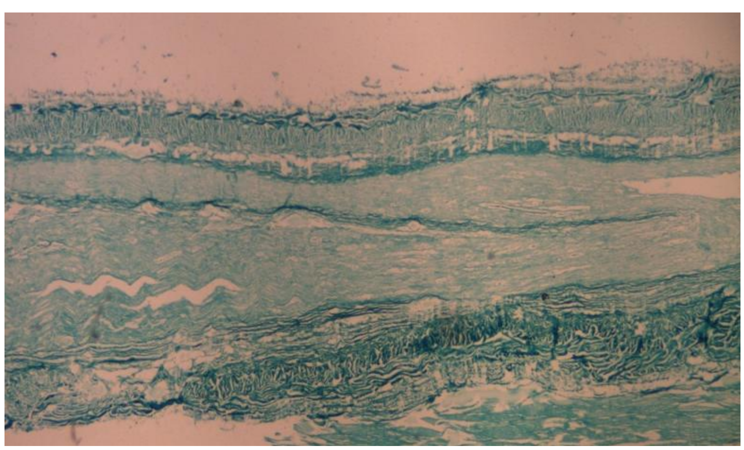


Figure 3: Section of the Sciatic Nerve in Group C. Upper and Lower Connective Tissue Show Perineurium (LuXAL FAST BLUE, 40)

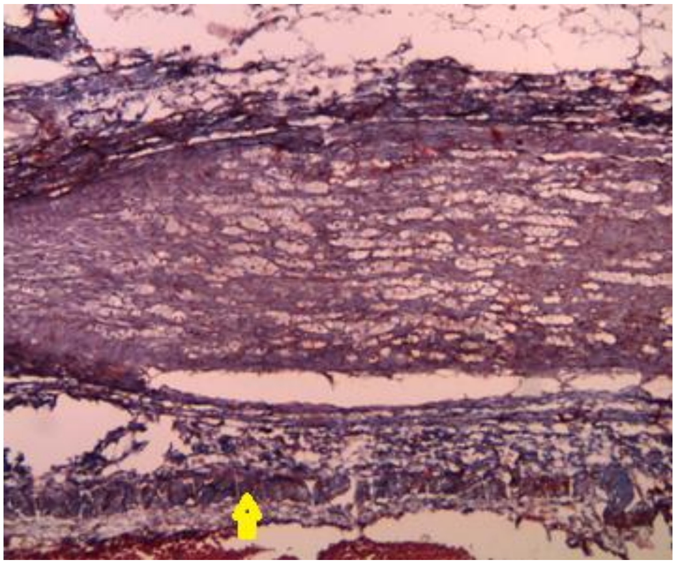


Figure 4: Arrow Shows Perineurium in Group c (Masson's Trichorome, 200x)

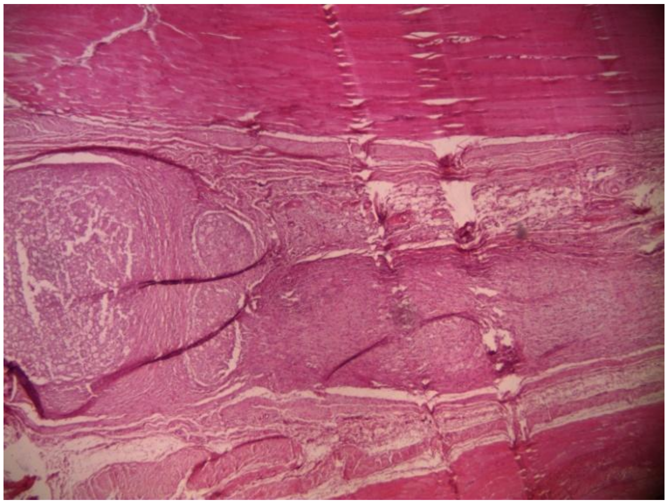


Figure 5: Regeneration Doesn't Happen in the Nerve in Group A (H&E, 40x)

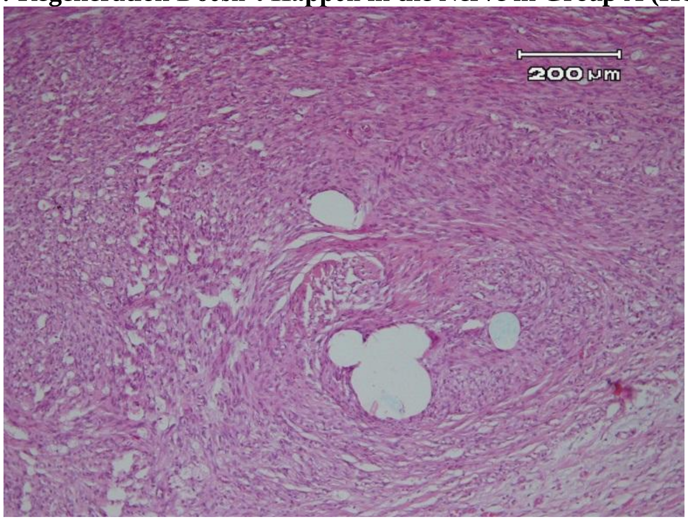
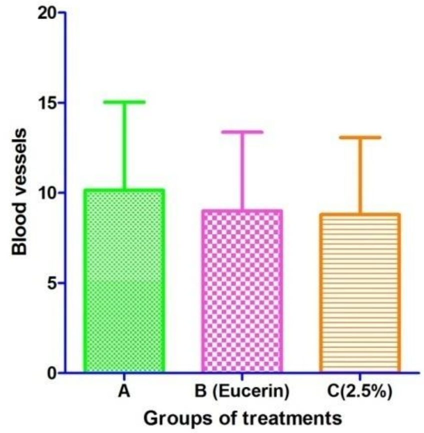
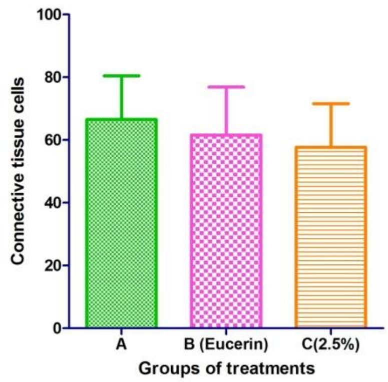


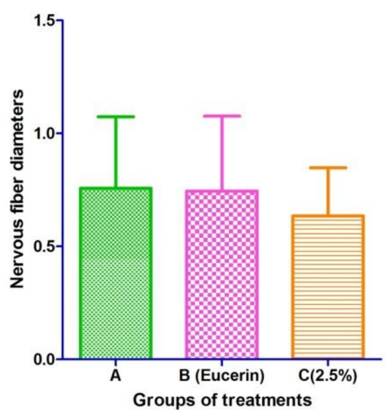
Figure 6: In Group B Like Group A the Ends of Nerve Don't Attach Each Other (H&E, 100x)



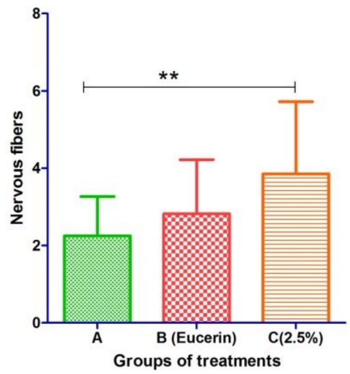
Statistical analysis of number of blood vessels between groups of treatments by Kruskal-Wallis and Dunn's Multiple Comparison Post-Test. mean±SD. There is no significant differences between groups.



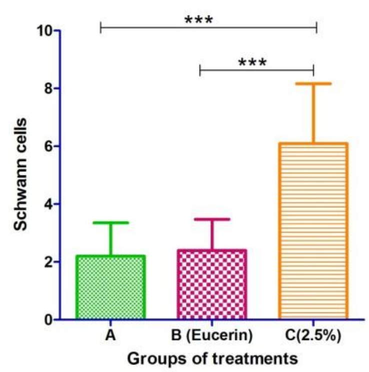
Statistical analysis of connective tissue cells between groups of treatments by Kruskal-Wallis and Dunn's Multiple Comparison Post-Test. mean±SD. There is no significant differences between the groups.



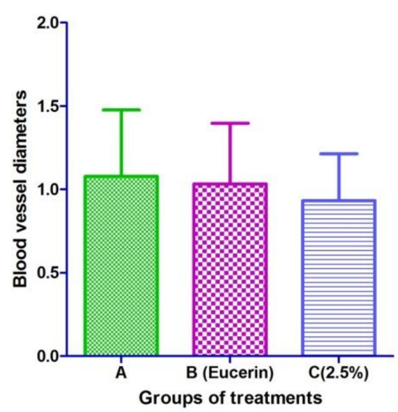
Statistical analysis of nervous diameters between groups of treatments by Kruskal-Wallis and Dunn's Multiple Comparison Post-Test. mean±SD. There is no significant differences between groups.



Statistical analysis of number of nervous fibers between groups of treatments by Kruskal-Wallis and Dunn's Multiple Comparison Post-Test. mean±SD. **P<0.01 indicate significant differences between groups.



Statistical analysis of Schwann cell numbers between groups of treatments by Kruskal-Wallis and Dunn's Multiple Comparison Post-Test. mean±SD. ***P<0.001 indicates significant differences between groups.



Statistical analysis of blood vessel diameters between groups of treatments by Kruskal-Wallis and Dunn's Multiple Comparison Post-Test. mean±SD. There is no significant differences between groups.

DISCUSSION

This experimental study compared Commiphora myrrha in an experimental repair technique in rabbit: the placebo and Commiphora myrrha oil known as myrrh, which has a regenerative effect on nerve repair. In present study, we used Commiphora myrrha. Commiphora myrrha is one of a physical barrier and to be effective in limiting adhesion to surgical incisions when it is used alone. Our findings suggest that the animals treated with 2.5% Commiphora myrrha developed lesser amounts of tissue inflammation. The findings establish the superiority of Commiphora myrrha over placebo repair with regards to the adhesion, fibroplasia, inflammation and nerve fiber regeneration. There were significant differences between the groups in the proliferation and organization of Schwan cells and induction and conduction of nerve defect ($P<0.05$). They were significantly lower in the control and placebo groups (group A and B) than Commiphora myrrha treated groups especially 2.5% Commiphora myrrha (group C). In this study, rabbit underwent a tension-free repair with a Commiphora myrrha return to their usual activities sooner than those underwent a non Commiphora myrrha tension-free repair. In group C, proper regeneration of Schwan cells of sciatic occurred and nerve fiber ingrowth was relatively completed with no evidence of a high degree of adhesion formation by the end of the experiment (45 days post-surgery). This study confirmed advantages of Commiphora myrrha to placebo. In this study, Commiphora myrrha was better when compared with the control group and placebo group. Less adhesion, no dehiscence, no recurrence and finally perfect healing occurred when Commiphora myrrha was used. The statistical analyses of results of pathological experiments suggest effective of commiphora myrha was 2.5% which applied to other groups because of significant ($P<0.05$) changes in connective tissue cells, Schwann cells and number of nervous fibers in comparison to control group. Eucerin did not make any significant changes in pathological aspects that we studied. On the basis of our results, we recommend using of the Commiphora myrrha to the repair of sciatic nerve defect, and we recommend that the Commiphora myrrha be used on the surrounding tissue. Also, infection did not lead to the damage of nerve repair in this study but it was a risk factor for recurrence. Therefore, the administration of broad-spectrum antibiotics at the induction of anesthesia is recommended. Our findings suggest that rabbit underwent Commiphora myrrha repair had fewer postoperative complications, muscular and vascular injuries and appeared to have less discomfort and to return to their usual activities more rapidly. Rabbit in the Commiphora myrrha -repair groups especially 2.5% Commiphora myrrha group had a better rate of healing than other groups.

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