

Effect of *Ricinus Communis* Aqueous Leaf Extract on the Healing of Wound. An in Vitro Study in the Cell Line

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Abstract

Background: Wound is characterized as harm or disturbance to the typical anatomical organization and function. As a result, muscles, nerves, arteries, tendons, parenchymal organs, and even bone may be harmed. It can also cause a basic breach in the tissue's epithelial integrity, such as dermal tissue, alternatively it may extend farther deeply into the subcutaneous tissue. *Ricinus communis* is a plant belong to the vast and significant family of flowering plants known as the Euphorbiaceae, different parts of the plant have bioactivity against a variety of diseases, include diabetes, wound infections, constipation, paralysis, and discomfort. There are a variety of phyto-constituents present, which contributes to these medicinal applications, includes reducing sugars, poly-uronides, glycosides, terpenoids, flavonoids, alkaloids, tannins, saponins, and steroids.

Aim of the study: To look into the impact of *Ricinus Communis* aqueous leaf extract on wound healing in MDCK cell line.

Materials) and (Methods: vertical wound was made by using 200µl sterile plastic micropipette tip to press firmly against the cell monolayer of tissue culture plate. Then the wounded MDCK cell was exposed to different concentrations of *Ricinus communis* aqueous leaf extract (500, 250, 125, 62.5, 31.25, 15.6) µg/ml.

Results: A) the concentration 125 µg/ml of *R. communis* aqueous extract caused significant increase in the cell viability percentage ($p \leq 0.05$) of the MDCK normal Cell line B) all concentrations of *Ricinus communis* aqueous leaf extract caused highly significant decrease ($p < 0.001$) in the diameter of the MDCK normal cell line induced wound after 3,6,9 hours of incubation and wound healing was completed after 12 hours of incubation.

Conclusion: On the MDCK cell line, all concentrations of *R.communis* leaf aqueous extract have wound healing effect.

Keywords: *Ricinus Communis*, Wound Healing, Normal MDCK Cell Line.

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INTRODUCTION

Wound is characterized as harm or disturbance to the typical anatomical organization and function. As a result, muscles, nerves, arteries, tendons, parenchymal organs, and even bone may be harmed. It can also cause a basic breach in the tissue's epithelial integrity, such as dermal tissue, alternatively it may extend farther deeply into the subcutaneous tissue (1) (2). Wound is produced by any incident involving a cut caused by a sharp object. It may result from the exploitation of the tissues' physical, chemical, thermal, microbial, or immune systems (3).

Healing of wounds is a normal physiological process that

happens in response to tissues' structural injury, including damage to the skin. Healing of wounds comprises four successive and overlapping stages including hemostasis, inflammation, proliferation (re-epithelialization) and remodeling (scar maturation) (4) (5).

Ricinus communis is a plant belong to the vast and significant family of flowering plants known as the Euphorbiaceae (6), it is spread in most of the world's tropical and subtropical regions. Typically, it is a little, soft-wooded tree that can reach a height of 6 meters. And they are extended to central America, India and Africa (7). Extracts from various parts of the plant have recently demonstrated remarkable bioactivities against a variety of

diseases, include pain, diabetes, wound infection, paralysis and constipation. It is also effective as an antioxidant, anti-inflammatory, hepato-protective and anticancer agent, owing to the presence of a diverse array of phyto-constituents such as tannins, glycosides, saponins, terpenoids, poly-uronides, flavonoids, steroids, alkaloids, anthrax-quinones and reducing sugars.

MATERIALS AND METHODS

Fresh leaves of *R. communis* plant were collected from Al-bakarly, in the city of Hilla – Iraq. Then were washed thoroughly with distilled water and allowed to dry for 14 days in a dark place at room temperature, then grinded to fine powder using electrical grinder and kept in a tightly sealed dry container for future use. Leaf powder then soaked and macerated in aqueous in concentration of 20 g of leaf powder in 200ml of DW and left for extraction at room temperature for 48 hours. Preparation of stock solution for aqueous extracts of *R. communis* was made by dissolving 60 mg of aqueous extract in 30 ml DDW to obtain final concentration of 2000 µg/ml and from this stock a serial dilution was made (1000, 500, 250, 125, 62.5, 31.25) µg/ml. This study include two parts:

Part one Cytotoxicity Assay: MDCK cell line was seeded in tissue culture 96-well plates and treatment with serial dilution of aqueous leaf extract (1000, 500, 250, 125, 62.5, 31.25) µg/ml and incubated at 37°C for 24 hours, then, the MTT assay was performed. To determine a range of

concentrations to be used for wound healing assay.

Part Two Wound Healing Assay: A vertical wound was made by using 200µl sterile plastic micropipette tip to press firmly against the cell monolayer of tissue culture plate. The injured cells were then exposed to various concentrations of *R. communis* aqueous leaf extract (500, 250, 125, 62.5, 31.25, 15.6) µg/ml.

After that the wound area were digitally photographed every 3 hours starting from 9 ante meridiem (0 hours), 12, 3, 6, and 9 post meridiem respectively. By using software “image J” (National Institutes of Health, Maryland, USA).

Microsoft Office Excel 2010 was used to collect and analyze all of the data. A one-way Anova test was used to investigate the differences between each treated group and the (control group). P values ($p \leq 0.001$, $p \leq 0.05$) were considered statistically significant.

RESULTS

1. Effect of Ricinus communis aqueous leaf extract on the viability of MDCK normal cell line

In comparison to the control group result showed insignificant difference ($p > 0.05$) in cell viability percentage of the MDCK normal Cell line treated with all concentrations of *R. communis* aqueous extract, except the concentration 125 µg/ml which caused significant increase in cell viability percentage ($p \leq 0.05$) of the MDCK normal Cell line (figure 1).

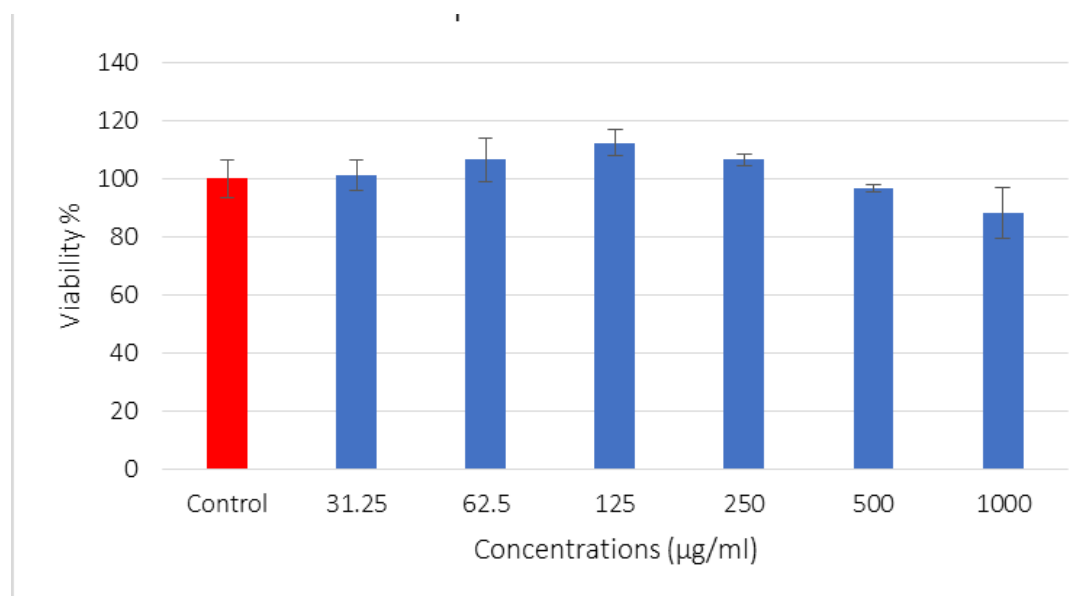


Figure 1: Effect of Ricinus communis aqueous leaf extract on the viability of MDCK normal cell line

2. Effect of Ricinus communis aqueous leaf extract on the healing of MDCK normal cell line- induced wound

In comparison to the control group results showed that all

concentrations of *R. communis* aqueous leaf extract caused highly significant decrease ($p < 0.001$) in the diameter of the MDCK normal cell line induced wound after 3,6,9 and 12 hours of incubation and wound healing was completed after

12 hours of incubation (Figure 2).

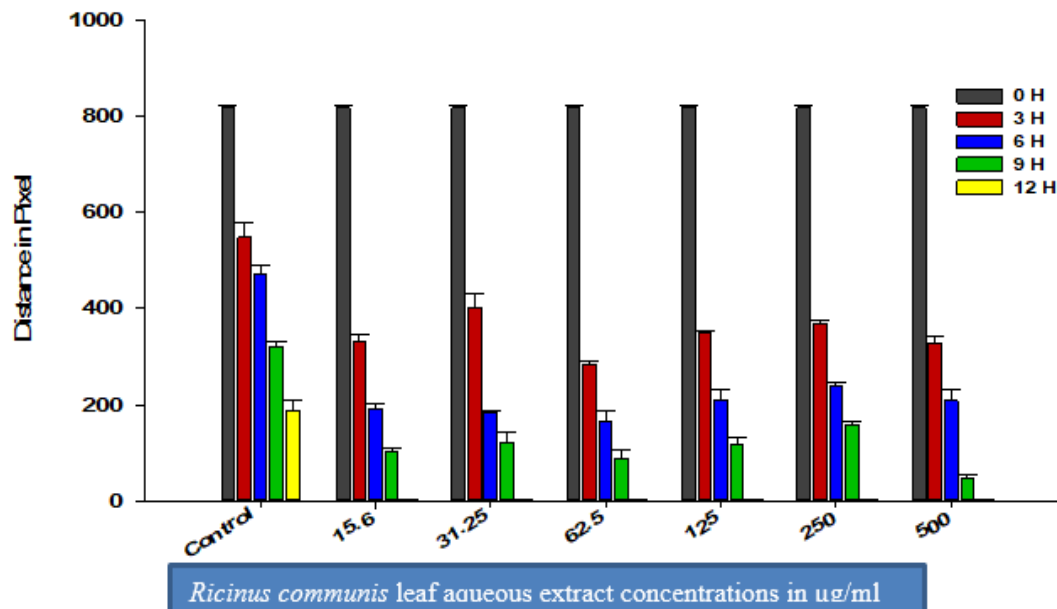


Figure 2: Effect of Ricinus communis aqueous leaf extract on the healing of MDCK normal cell line- induced wound

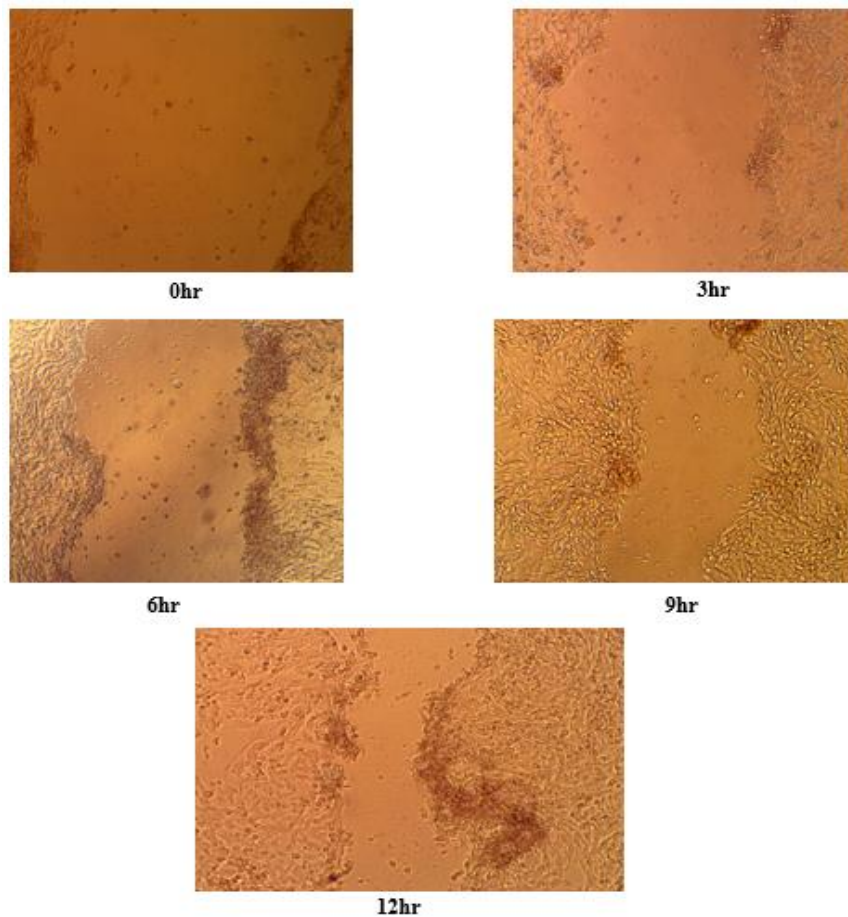


Figure 3: Inverted Microscope (10X Lence) Image of MDCK cell line without treatment of Ricinus communis aqueous leaf extract (control group) after incubation for 12 hour

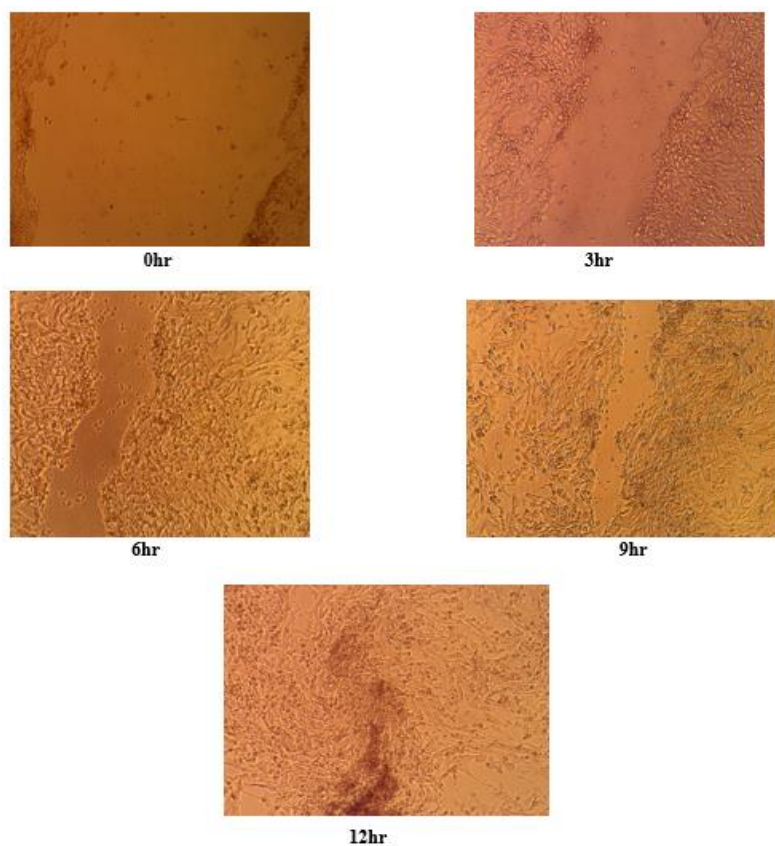


Figure 4: Inverted Microscope (10X Lence) Image of MDCK cell line treated with Ricinus communis aqueous leaf extract (15.6 µg/ml) after incubation for 12 hours

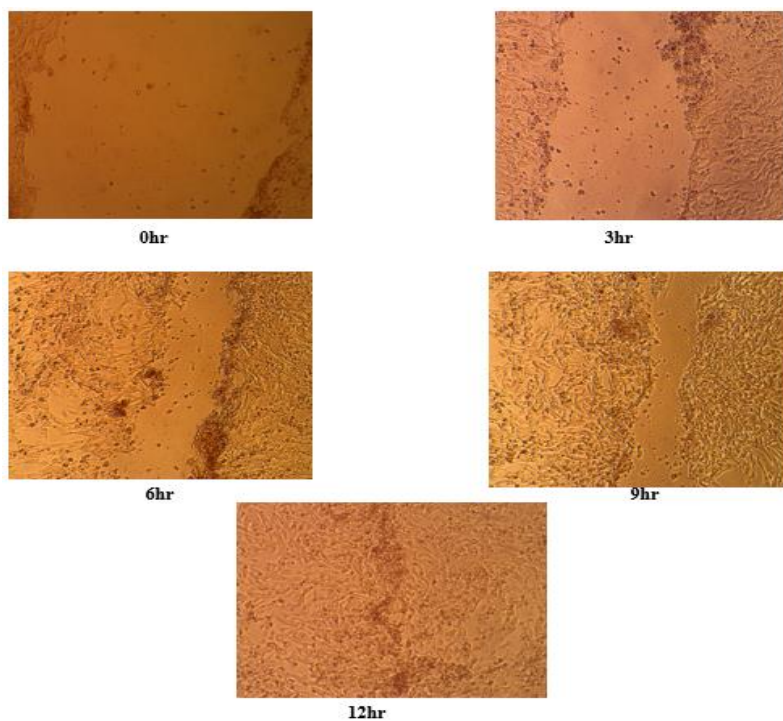


Figure 5: Inverted Microscope (10X Lence) Image of MDCK cell line treated with Ricinus communis aqueous leaf extract (31.25 µg/ml) after incubation for 12 hours

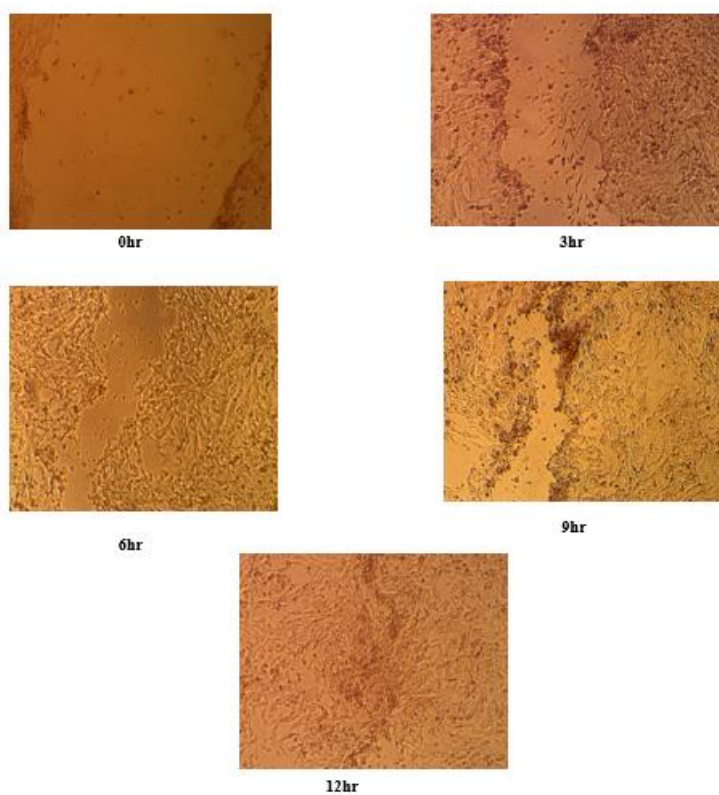


Figure 6: Inverted Microscope (10X Lence) Image of MDCK cell line treated with Ricinus communis aqueous leaf extract (62.5 µg/ml) after incubation for 12 hours

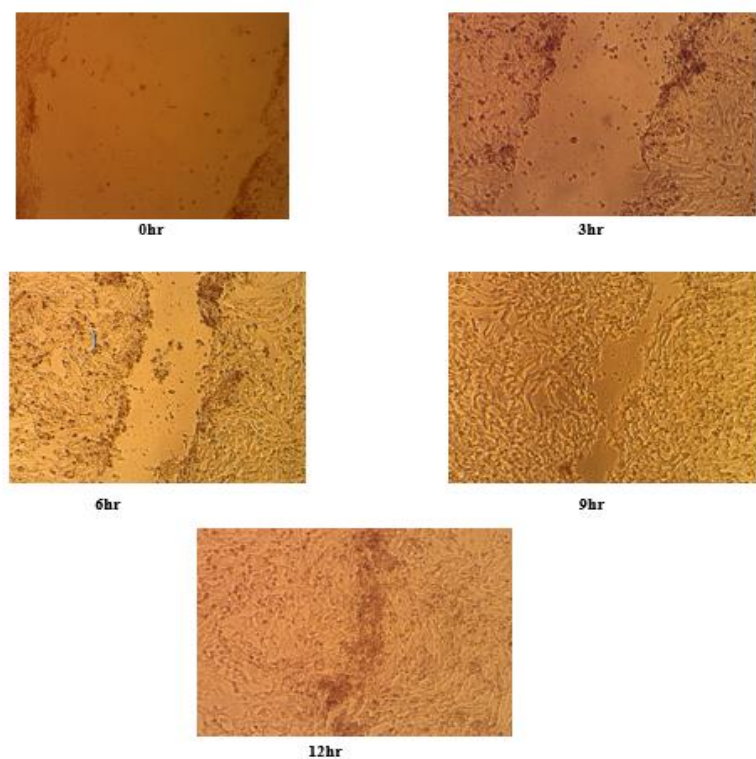


Figure 7: Inverted Microscope (10X Lence) Image of MDCK cell line treated with Ricinus communis aqueous leaf extract (125 µg/ml) after incubation for 12 hours

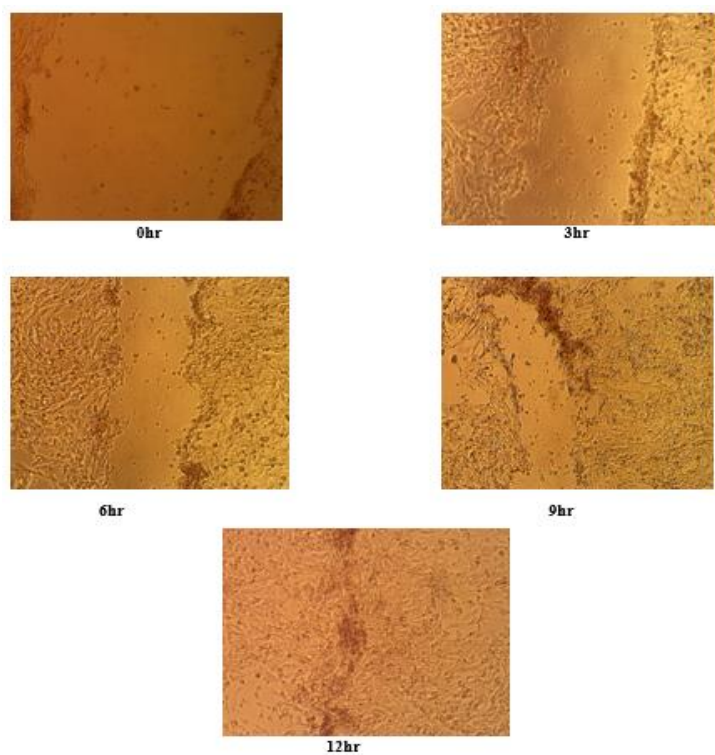


Figure 8: Inverted Microscope (10X Lence) Image of MDCK cell line treated with Ricinus communis aqueous leaf extract (250 µg/ml) after incubation for 12 hours

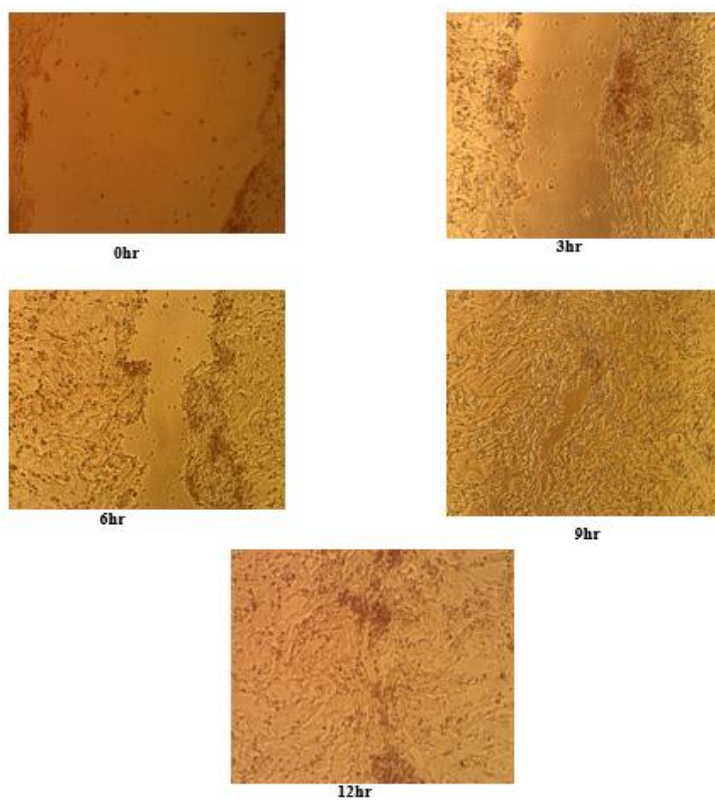


Figure 9: Inverted Microscope (10X Lence) Image of MDCK cell line treated with Ricinus communis aqueous leaf extract (500 µg/ml) after incubation for 12 hours

DISCUSSION

1. Effect of Ricinus communis aqueous leaf extract on the viability of MDCK normal cell line

In the present study the increase in the viability of MDCK normal cell line after treated with 125 µg/ml of *R. communis* aqueous leaf extract agree with that reported by Mohamed *et al.*, (2019) study which revealed that both two extracts (aqueous-ethanol and ethanol) of *R. communis* leaf extract given by the oral route was safe up to a dose of 2,000 mg/kg/of body weight, and did not cause any mortality or toxic effects in the treated animals' behavior.

Another study by Vhutshilo and Peter, (2014) disagree with that results which revealed that the low concentrations of *Ricinus communis* (acetone, dichloromethane, hexane, and methanol) leaf extracts had minimal toxicity on Bud-8 cell line, while at high concentrations the extracts are highly toxic. Cell viability decreased as concentrations increased of *R. communis* alcoholic leaf extracts. The leaves of *R. communis* contain ricin, but only in trace amounts. Ricin has been shown to be highly toxic to mammalian cells. It kills cells by interfering with protein synthesis.

2. Effect of Ricinus communis aqueous leaf extract on the healing of MDCK normal cell line-induced wound

In the present study the decrease in the diameter and complete healing of the MDCK normal cell line-induced wound after the treatment with *R. communis* aqueous leaf extract at all concentrations may be attributed to the presence of steroids, alkaloids, tannins, phenols, saponins, phytates, oxalates flavonoids and glycosides in different parts of the plant including leaves as all these compounds have antimicrobial properties (Rashmi *et al.*, 2019).

Moreover it had been found that the antimicrobial properties of phyto-chemical compounds that are present in different parts of *R. communis*, promote the wound healing process, as they enhance wound contraction and increase rate of epithelialization (Sonali and Shonkor, 2015).

The high effectiveness of aqueous *R. communis* leaf extract reported by the present study is disagree with that found by Rabia and Asghari, (2012) study which revealed that the aqueous extract of leaf had low activity against both fungal and bacterial strain as compared to methanol extracts and explaining this result by the solubility of most phytochemical compounds which having antimicrobial potential in the methanolic more than aqueous solvents.

The wound healing enhancement of the *R. communis* aqueous leaf extract by the present study could related to the presence of flavonoids: rutin, quercetin, epicatechin and polyphenols (Gallic and ellagic acid) and gentesic acid, these compounds which are potent anti-oxidant and anti-inflammatory agents (Anil *et al.*, 2010). Inflammation is the

primary wound-related characteristics caused by the release of prostaglandins, leukotriene, eicosanoids and reactive oxygen species, thus compounds such as flavonoids which is one of the key bioactive sources found in medicinal plants, and it has a wide range of pharmacological properties, such as antioxidant (free radical scavenger), antimicrobial, hepato-protective, anti-inflammatory, anticancer activities, growth regulators could play an important role as wound healing enhancer Muhammad *et al.*(2018).

CONCLUSION

All concentrations of *R. communis* aqueous leaf extract have wound healing effects on the MDCK wounded cell line.

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