Original article

A Dose Optimization Study of *Marantodes pumilum* (Blume) Kuntze Extracts for Wound Healing Effect in Animal Model

Shihab UA[1], Atiqah A[2], Ahmad NS[3], Isa NM[4]

Abstract

Objective: This study was done in the ovariectomized rat model to optimize the dose of*Marantodespumilum* (MP) for the treatment of open wound healing activities. ***Materials and Methods:*** Total fifty-six rats were taken for this study which was split into four groups based on four extracts such as *Marantodes pumilum* var. *pumila* (MPvp) leaf and root extracts and *Marantodes pumilum* var. *alata* (MPva) leaf and root extracts; each group contains fourteen rats. Each group was further divided into seven subgroups with equally distributed rats which consisted with one control group without treatment (Control) and six treated groups with different concentrations: 0.1% Conc, 0.5% Conc, 1.0% Conc, 2.0% Conc, 3.0% Conc and 4.0% Conc of each MP extract. After punch biopsy, rats were treated with ointment prepared by cetomacrogol emulsifying agent and MP extract on daily basis, beginning from the wound creation until healed completely. The parameters studied were the macroscopic observation andthe wound area measurement. ***Results:*** Wounds with treated 1.0% and 4.0% Conc of MPvpleaf and root extracts and 2.0%, 3.0% and 4.0% Conc of MPvaleaf and root extracts were healed completely at day 9, whereas wounds of control groups were healed at day 12 for each extract group. The wound area was significantly reduced in the treated groups with 1.0% and 4.0% Conc ofMPvpleaf and root extracts ($p < 0.05$) and 2.0%, 3.0% and 4.0% Conc of MPvaleaf and root extracts ($p < 0.05$) than their respective control group. ***Conclusion:*** The dose of 1.0% concentration for both leaf and root extracts of MPvp and 2.0% concentration for both leaf and root extracts of MPva exhibited the best effect to expedite for open wound healing compared to control group and other concentrations in the ovariectomized rat model.

Keywords: *Marantodes pumilum*; wound size; wound healing; ovariectomized

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Introduction

Medicinal plants have been used extensively as remedies for human health since ancient times due to its therapeutic efficacy associated with their purified constituents. Experimental investigations demonstrated that plant extract has many biological properties such as antioxidant, antimicrobial and anticancer effects revealed by bioassay systems and

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animal models[1,2]. MP is also medicinal plants widely found in south-east Asia which previously named as *Labisia pumila* (Blume) Fern-Vill[3]. It is a member of Primulaceae familywhich featured as leafy plant with creeping stem. The leaves are in lanceolate shape and size can be up to 30 cm long and 13 cm wide. Some species have also pink color flowers and red to purple color fruits. It is one of the most popular medicinal plants in Malaysia[3]. MP is locally known as kacip Fatimah in Malaysia. There are many variants of MP including *var. pumila* and *var. alata* found widely in Malaysia[4]. These species can easily differentiate by their stem, petiole and leaf[5]. In Malaysia, MP is a well-known medicinal plant that has long been recognized and much demanded value as tonics and health products[6]. Malay women are consumed during their pregnancy to become ease child delivery. It is to believe that MP can also help to recover from menstruation related difficulties, to regain the body stamina, to relieve weakness and alleviate menopausal symptoms[7]. It is drunk traditionally by boiling the whole plants. Other traditional uses include to alleviate dysentery, rheumatism, and gonorrhea[8]. It can also maintain of abdominal muscle tonicity by reducing excessive fat[8], reduce the risk of osteoporosis[10], metabolic disorders[11] and cardiovascular diseases[12]. Several studies have been conducted to identify and characterise the bioactive phytochemicals from MP. The results showed that the plant has numerous pharmacological activities including phytoestrogenic, anti-oxidant, anti-inflammatory, anti-fungal, anti-microbial and anti-aging effects[13,14]. These biological properties should play an important role in the different stages of wound healing and should expedite the process[15]. This study investigates and evaluate the optimum dose concentration of MP aqueous extract formulated as an ointment for wound healing.

Materials and Methods

Extraction of MP and Ointment Preparation

After collecting of MP from local hill track in Malaysia, the plants were authenticated and separated into two species including MPvp and MPva. Four aqueous extracts were obtained from the plantsby the standardized extraction method[13]. In brief, separated plant materials into leaf and root were thoroughly washed and dried at 40 ºC for three days. Dried materials were ground and weighted before use. Plant materials were dissolved in distilled water (1:10) and boiled at 60 ºC for twohrs with continuous stirring. After boiling the water, thesolvent was filtered and placed in the freezer at -80ºC. Then it was lyophilized by freeze dryer to produce dried powder stock. The dried powder stock obtained was a dark brown powdery extract. The average yield was around 8-10%. To prepare the ointment, each extract was grinded into powder by using mortar and pestle and weighted for six different concentrations such as 0.1%, 0.5%, 1.0%, 2.0%, 3.0% and 4.0% of each extract powder and mixed uniformly with vehicle (Cetomacrogol,HovidBerhad, Malaysia).

Animal Handling and Tropical treatment

56 female Sprague-Dawley rats (200–250 g, 3–5 months old) obtained from animal house of Universiti Kebangsaan Malaysia. They were housed in cages for a week to acclimatize the lab environment. After ovariectomy, rats were observed at least two weeksfor estrogen deficiency state[16]. Mixed solution of ketamine (50 mg/ml) and xylazine (20 mg/ml) as 1:1 ratio was used as anesthetic agent. Rats were anesthetized intraperitoneally during all surgical procedures[17]. Rats were then divided into four different groups based on *Marantodes pumilum* extracts; each group contained 14 rats. Each group was further divided into 7 subgroups with equally distributed rats which consisted with one control group without treatment (Control) and six treated groups with varying concentrations: 0.1% Conc, 0.5% Conc, 1.0% Conc, 2.0% Conc, 3.0% Conc and 4.0% Conc of *Marantodes pumilum* extract. The dorsal surface of rats was shaved with a sterile razor blade and disinfected with 70% alcohol. 6 mm diameter punch biopsy was used to make wounds. Four wounds were made bilaterally on dorsal surface of each rat. The treatment ointment was given topically once daily until complete healing of all wounds.

Macroscopic Observation and Wound Contraction Measurement

The wound healing was examined by two factors including macroscopic observation and the measurement of wound contraction for the

preliminary study. To examine wound contraction and macroscopic view, the wound area was calculated and taken photograph of injury area of skin on day zero and following day on 1, 3, 6, 8, 9, 10, 11 and 12 after injury. Digital caliper was used for calculating wound area referred by clock method[18].

Statistical Analysis

Data are presented as means ± SD. SPSS (23version) statistical software is used for statistical analysis. The effect of extractsvs healed day of wounds were compared using one-way analysis of variance (ANOVA). *P* values less than0.05 were considered significant.

Ethical Issue

The study approved by Universiti Kebangsaan Malaysia Animal Ethics Committee (ethical approval number: FP/FAR/2014/ISA/26-NOV./637-JAN.-2015-DEC.-2016).

Results

Wounds Macroscopic Observation

The figure1 shows the macroscopic view of wounds for four groups: MPvp leaf and root extract and MPva leaf and root extract groups respectively. Each group was subdivided into seven groups; one control group and six treated groups with six different concentrations: 0.1%, 0.5%, 1.0%, 2.0%, 3.0% and 4.0% of the extract. In the microscopic view, wounds treated with 1.0% Conc and 4.0% Conc of MPvp leaf and root groups healed on day 9. While rest of groups with another different concentration of MPvp leaf and root extracts such as 0.1% Conc, 0.5% Conc, 2.0% Conc and 3.0% Conc healed slower at 10 or 11 days.In MPva leaf and root extract groups, the wounds treated with 2.0% Conc, 3.0% Conc and 4.0% Conc of MPva leaf and root extract healed on day 9, whereas, wounds treated with 0.1% Conc, 0.5% Conc and 1.0% Conc healed slower. Wounds for the control group in all four groups healed the slowest at day 12. Although there were a few concentrations which healed the earliest at same time, the lowest concentration was chosen as the optimum concentration for each extract. Therefore, the concentration of 1.0% was chosen for MPvp root and leaf extract and 2.0% was chosen for MPva leaf and root extract to be the optimum concentration for wound healing.

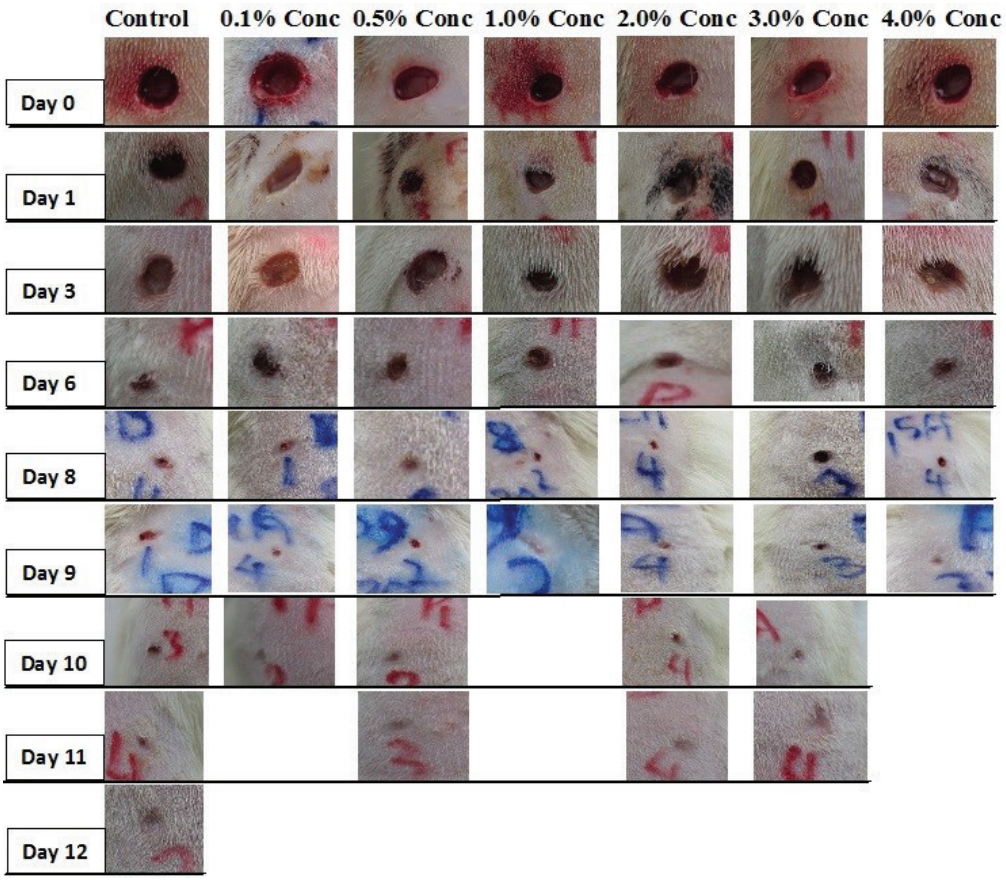
Determination of Wound Contraction

Figure 2 shows the mean value of complete wound healed day for four groups; MPvp leaf and root extract and MPva leaf and root extract. Each group was subdivided into seven groups: control group and six treated groups with 0.1%, 0.5%, 1.0%, 2.0%, 3.0% and 4.0% concentration respectively. The bar charts illustrate the wounds treated with 1.0% Conc and 4.0% Conc of both MPva leaf and root extract groups healed faster compared to all other concentrations (0.1% Conc, 0.5% Conc, 2.0% Conc and 3.0% Conc). On the complete healed day, wounds treated with 1.0% Conc and 4.0% Conc of both MPva leaf and root extract groups showed significantly faster healing than the control group ($p< 0.05$). In the MPva leaf and root extract groups, wounds treated with 2.0% Conc, 3.0% Conc and 4.0% Conc showed significantly faster healing than the control group ($p< 0.05$). There were no significant differences between other treated groups and control group or in between treated groups.

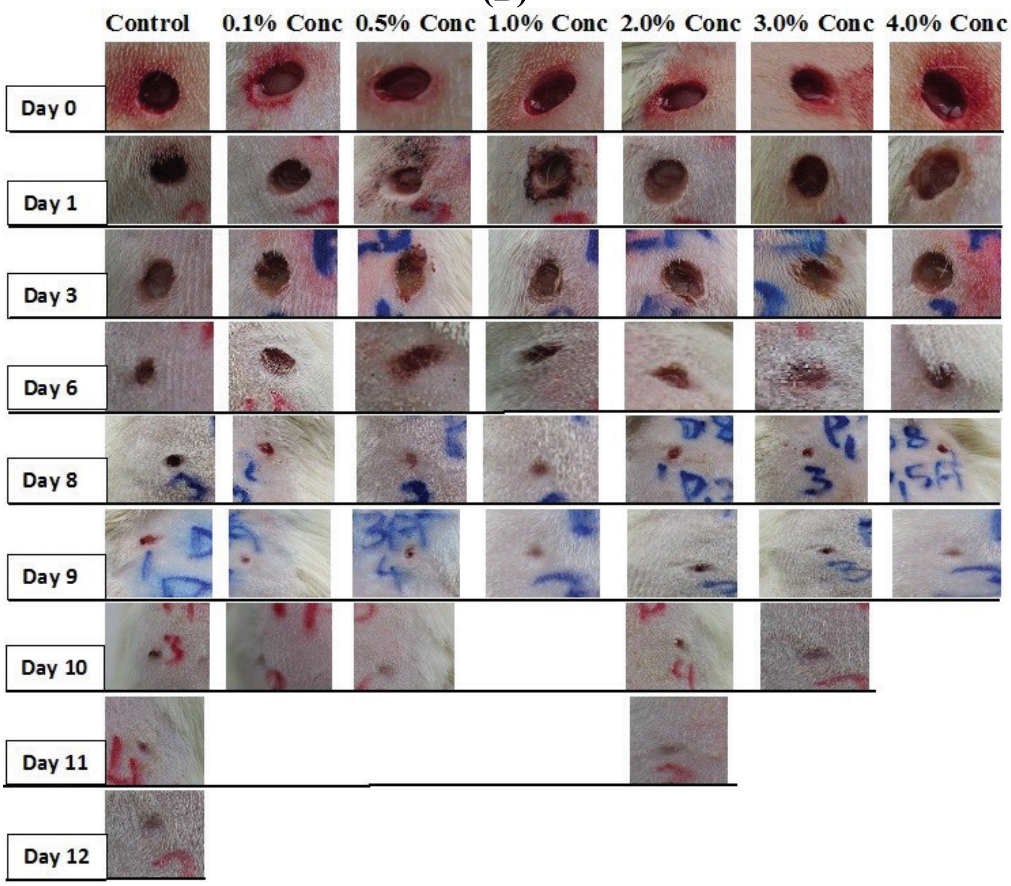
Table 1 demonstrate the mean area (mm^2) of wound determined for control and six treated groups with different concentrations of four extract group against treatment day 0, 1, 3, 6, 8, 9, 10, 11 and 12. The rate of contraction of the wound area of 1.0% and 4.0% Conc groups was higher compared to the other groups. On day 9, wound contraction area of 1.0% and 4.0% Concof MPvp leaf and root extract groups was 0 mm^2 (completely closed). In the MPva leaf and root extract groups, wound contraction area of 2.0%, 3.0% and 4.0% Conc was 0 mm^2 (completely closed) on day 9.

Table 1. The mean wound area (mm2) of control and six treated groups with different concentrations: 0.1% Conc, 0.5% Conc, 1.0% Conc, 2.0% Conc, 3.0% Conc and 4.0% Conc for each extract; (A) MPvp leaf extract, (B) MPvp root extract, (C) MPva leaf extract and (D) MPva root extract against treatment day 0, 1, 3, 6, 8, 9, 10, 11 and 12. All data are given as mean ± S.E. for two animals in each group.

(A)


Control
0.1% Conc
0.5% Conc
1.0% Conc
2.0% Conc
3.0% Conc
4.0% Conc
Day 0
Day 1
Day 3
Day 6
Day 8
Day 9
Day 10
Day 11
Day 12

(B)


Control
0.1% Conc
0.5% Conc
1.0% Conc
2.0% Conc
3.0% Conc
4.0% Conc
Day 0
Day 1
Day 3
Day 6
Day 8
Day 9
Day 10
Day 11
Day 12

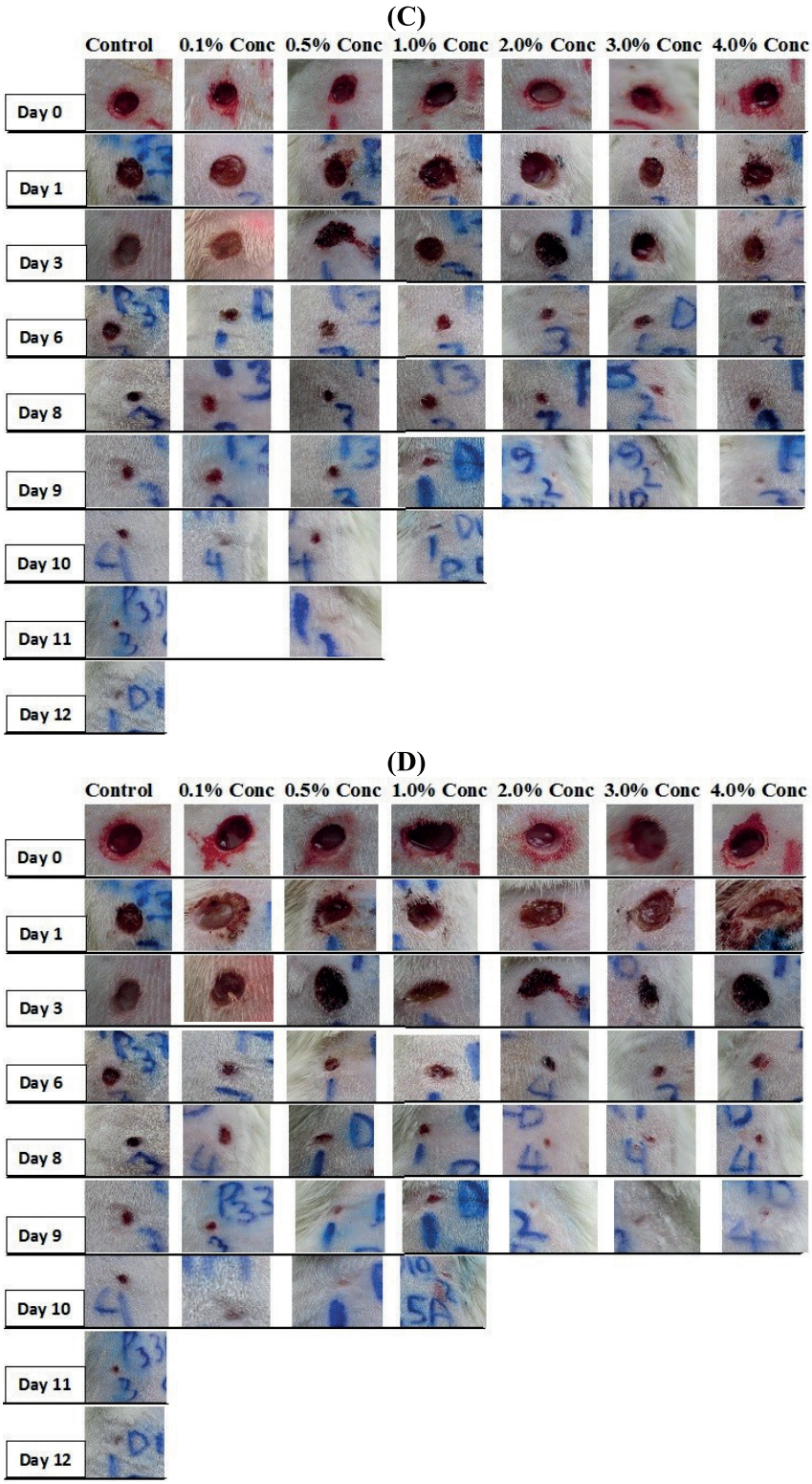


Figure 1. Macroscopic view of wounds of control and six treated groups with different concentrations: 0.1% Conc, 0.5% Conc, 1.0% Conc, 2.0% Conc, 3.0% Conc and 4.0% Conc of each extract; (A) MPvp leaf extract, (B) MPvp root extract, (C) MPva leaf extract and (D) MPva root extract against treatment day 0, 1, 3, 6, 8, 9, 10, 11 and 12.

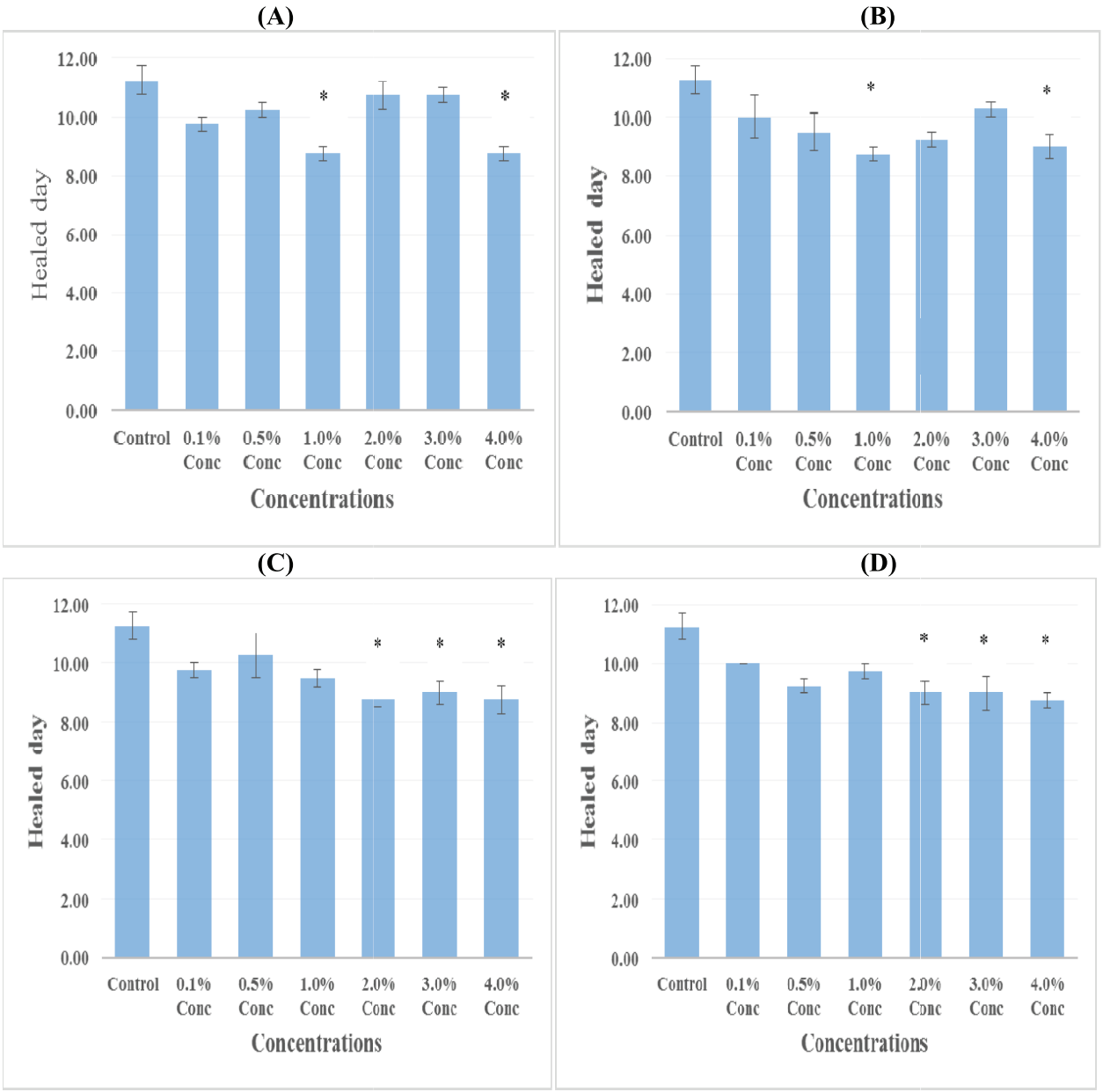


Figure 2. Bar chart represents the wounds healed day forcontrol and six treated groups with different concentrations: 0.1% Conc, 0.5% Conc, 1.0% Conc, 2.0% Conc, 3.0% Conc and 4.0% Concof each extract; (A) MPvp leaf extract, (B) MPvp root extract, (C) MPva leaf extract and (D) MPva root extract. All data are given as mean ± S.E. for two animals in each group. Statistically significant results indicated as (*)$p < 0.05$ versus thecontrol group.

Groups	Day 0	Day 1	Day 3	Day 6	Day 8	Day 9	Day 10	Day 11	Day 12
Control	28.27 ± 0.00	26.04 ± 2.12	22.46 ± 1.58	7.80 ± 1.21	2.70 ± 0.44	0.86 ± 0.25	0.44 ± 0.23	0.29 ± 0.23	0.00 ± 0.00
0.1% Conc	28.27 ± 0.00	25.47 ± 1.50	24.00 ± 1.64	10.58 ± 3.03	5.02 ± 0.25	1.33 ± 0.48	0.00 ± 0.00	-	-
0.5% Conc	28.27 ± 0.00	28.79 ± 1.30	21.71 ± 1.28	13.19 ± 2.61	5.16 ± 0.72	1.44 ± 0.49	0.23 ± 0.23	0.00 ± 0.00	-
1.0% Conc	28.27 ± 0.00	25.15 ± 1.63	24.15 ± 2.83	11.09 ± 1.27	0.90 ± 0.31	0.00 ± 0.00	-	-	-
2.0% Conc	28.27 ± 0.00	29.77 ± 0.39	24.37 ± 1.46	15.20 ± 2.58	8.10 ± 1.39	3.71 ± 1.37	1.15 ± 0.70	0.20 ± 0.20	0.00 ± 0.00
3.0% Conc	28.27 ± 0.00	33.95 ± 2.41	18.61 ± 2.15	9.59 ± 1.97	3.88 ± 0.49	2.21 ± 0.88	0.60 ± 0.25	0.00 ± 0.00	-
4.0% Conc	28.27 ± 0.00	27.96 ± 1.22	14.65 ± 0.11	3.29 ± 1.00	0.85 ± 0.39	0.00 ± 0.00	-	-	-

(B)

Groups	Day 0	Day 1	Day 3	Day 6	Day 8	Day 9	Day 10	Day 11	Day 12
Control	28.27 ± 0.00	26.04 ± 2.12	22.46 ± 1.58	7.80 ± 1.21	2.70 ± 0.44	0.86 ± 0.25	0.44 ± 0.23	0.29 ± 0.23	0.00 ± 0.00
0.1% Conc	28.27 ± 0.00	30.98 ± 1.48	24.47 ± 3.88	11.85 ± 2.18	3.31 ± 2.09	1.08 ± 0.89	0.32 ± 0.32	0.20 ± 0.20	0.00 ± 0.00
0.5% Conc	28.27 ± 0.00	27.58 ± 0.39	21.06 ± 1.51	7.81 ± 0.93	0.98 ± 0.56	0.13 ± 0.08	0.06 ± 0.06	0.00 ± 0.00	-
1.0% Conc	28.27 ± 0.00	28.26 ± 1.31	21.08 ± 2.15	9.05 ± 0.65	0.61 ± 0.47	0.00 ± 0.00	-	-	-
2.0% Conc	28.27 ± 0.00	26.90 ± 0.76	20.26 ± 4.03	7.72 ± 1.34	1.51 ± 0.13	0.09 ± 0.09	0.00 ± 0.00	-	-
3.0% Conc	28.27 ± 0.00	36.13 ± 2.56	21.95 ± 4.19	5.64 ± 1.90	2.95 ± 1.06	0.69 ± 0.18	0.22 ± 0.22	0.00 ± 0.00	-
4.0% Conc	28.27 ± 0.00	32.58 ± 1.25	21.89 ± 2.04	3.22 ± 1.29	1.19 ± 0.22	0.00 ± 0.00	-	-	-

(C)

Groups	Day 0	Day 1	Day 3	Day 6	Day 8	Day 9	Day 10	Day 11	Day 12
Control	28.27 ± 0.00	34.22 ± 2.51	28.25 ± 2.85	18.30 ± 4.59	6.63 ± 0.85	3.27 ± 1.02	0.69 ± 0.33	0.52 ± 0.34	0.00 ± 0.00
0.1% Conc	28.27 ± 0.00	23.65 ± 1.85	22.60 ± 1.35	14.85 ± 3.79	4.25 ± 0.72	0.52 ± 0.21	0.00 ± 0.00	-	-
0.5% Conc	28.27 ± 0.00	31.36 ± 1.66	24.84 ± 3.86	15.48 ± 2.50	4.55 ± 1.40	1.65 ± 0.95	0.73 ± 0.44	0.00 ± 0.00	-
1.0% Conc	28.27 ± 0.00	24.48 ± 2.71	25.79 ± 3.25	8.30 ± 1.36	3.28 ± 0.94	0.48 ± 0.29	0.00 ± 0.00	-	-
2.0% Conc	28.27 ± 0.00	30.73 ± 2.15	23.21 ± 4.32	9.97 ± 1.37	0.79 ± 0.35	0.00 ± 0.00	-	-	-
3.0% Conc	28.27 ± 0.00	23.07 ± 1.31	23.07 ± 1.31	8.40 ± 1.87	0.64 ± 0.31	0.00 ± 0.00	-	-	-
4.0% Conc	28.27 ± 0.00	22.30 ± 5.40	22.30 ± 5.40	5.27 ± 0.66	0.51 ± 0.31	0.00 ± 0.00	-	-	-

(D)

Groups	Day 0	Day 1	Day 3	Day 6	Day 8	Day 9	Day 10	Day 11	Day 12
Control	28.27 ± 0.00	34.22 ± 2.51	28.25 ± 2.85	18.30 ± 4.59	6.63 ± 0.85	3.27 ± 1.02	0.69 ± 0.33	0.52 ± 0.34	0.00 ± 0.00
0.1% Conc	28.27 ± 0.00	25.17 ± 1.16	21.37 ± 1.47	10.99 ± 1.13	2.44 ± 0.57	1.12 ± 0.48	0.00 ± 0.00	-	-
0.5% Conc	28.27 ± 0.00	32.19 ± 5.84	27.06 ± 5.54	10.69 ± 1.66	2.98 ± 1.22	0.28 ± 0.28	0.00 ± 0.00	-	-
1.0% Conc	28.27 ± 0.00	29.72 ± 2.30	27.53 ± 3.12	9.45 ± 2.26	1.18 ± 0.15	0.49 ± 0.17	0.00 ± 0.00	-	-
2.0% Conc	28.27 ± 0.00	24.94 ± 1.02	26.38 ± 0.79	10.88 ± 1.47	0.60 ± 0.36	0.00 ± 0.00	-	-	-
3.0% Conc	28.27 ± 0.00	21.94 ± 1.02	21.94 ± 1.02	7.20 ± 1.23	0.79 ± 0.46	0.00 ± 0.00	-	-	-
4.0% Conc	28.27 ± 0.00	20.11 ± 1.76	20.11 ± 1.76	8.31 ± 1.15	0.33 ± 0.15	0.00 ± 0.00	-	-	-

Discussion

In this study, the effects of MP were studied on the wound healing process in rats through the measurement of wound contraction and macroscopic observation of skin wounds. Results of this study demonstrated that aqueous extract MP ointment has the potential to expedite the wound healing process. Wounds treated with MP healed faster compared to normal wound healing process. This effect could be due to the many biological properties of MP.

MP contains many phytochemicals such as flavonoids, phenolic compounds, methyl gallate, alkaloids, alkenyl compounds, glycosides, tannins, phenolics, anthocyanins, sterols, triterpenoids, carotenoids, benzoquinone derivatives, ascorbic acids, saponins, and fatty acids[19,20]. Flavonoid which is also called polyphenol is another class of secondary plant metabolites. Many secondary metabolites were identified from MP as flavonoids and phenolic compounds including rutin, myricetin, apigenin, kaempferol, caffeic acid, gallic acid and pyrogallol[21]. Flavonoid and phenolic compounds are the secondary metabolites reported to have the most antioxidant potential[22]. Wounds make oxidative stress due to release of excessive molecular oxygen or free radicals which can delay of wound healing process. Antioxidants can enhance the healing rate by reducing these radicals at the wound site[23]. Recent studies reported that phytochemical constituents such as flavonoids, triterpenoids, and tannins could promote the wound-healing process as an anti-inflammatory agent[24-26]. Chen et al (2012)[27] studied on pro-inflammatory and anti-inflammatory activity with the medicinal plant. The findings of the study suggested that plant extract regulates the pro-inflammatory and anti-inflammatory cytokines which induce the systemic immune pathways to proliferate cells at the injury site. The MP methanolic extracts have antibacterial properties against both Gram-positive and Gram-negative pathogens[28]. MP contains fatty acids such as palmitic, palmitoleic, stearic, oleic, linoleic and α-linolenic[29]. The fatty acids can exhibit antibacterial activity[30].

There is no previous study of MP on wound healing effects. Therefore,the dose and duration optimization study of MP have to conduct for wound healing effect. However, many previous researchs of MP treated with various oral dosages were studied. Fazliana et al (2012)[31] studied on metabolic disorders in ovariectomized rats treated with MPva extracts at three different doses, 10 mg/kg/day, 20 mg/kg/day and 50 mg/kg/day. Effendy and Shuid [32] observed the inhibitory effects of MPva extracts on osteoporosis at 20 mg/kg/day and 100 mg/kg/day dosages. A minimal dose of 50 mg/kg of MP aqueous extract did not show any toxic effect on the reproductive system in female rats[33]. A study showed that histological abnormalities in lungs, kidney and liver with high levels of liver enzymes in rats after consuming between 200 to 1,000 mg/kg of MP extracts[34]. Treatment of non-pragnant rats by MP extracts showed a little histological changes in the endometrial wall thickness[35]. Mammalian bone marrow cells were assayed with treated of various dosages of MP aqueous extracts. There wasn't found any effect of mutation or genotoxicity in assay[36]. In this study, six different concentrations including 0.1%, 0.5%, 1.0%, 2.0%, 3.0% and 4.0% of MP in each extract were used topically with vehicle ointment to determine the best dose. Due to the nature of our

study using the topical application only, the amount used and absorbed is very minimal. The amount of ointment used for each wound is less than 1g, which is equivalent to 0.04g of MP extract at the highest concentration with 100% absorption. All doses used were safe according to the previous toxicity studies in rats.

Conclusions

The dose of 1.0% concentration for the two extracts of MPvp leaf and root and 2.0% concentration for MPva leaf and root extracts exhibited the best effect to expedite wound healing in rat animal model compared to control group and other concentrations.

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Conflict of Interest

No conflict of interests is declared by authors.

Author Contribution Statements

SUA carried out the experiment. SUA wrote the manuscript with support from AA, ANS and INM. INM and ANS supervised the project. INM conceived the original idea. All authors discussed the results and contributed to the final manuscript.

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