

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.608.373>

Wound Healing and Anti-Inflammatory Activity of Methanolic Extract of *Gmelina arborea* and *Hemigraphis colorata* in Rats

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ABSTRACT

Gmelina arborea and *Hemigraphis colorata* are traditionally used by tribal healers of Wayanad district, Kerala India. The wound healing and anti-inflammatory activity of *Gmelina arborea* and *Hemigraphis colorata* were tested in adult wistar albino rats. Wound healing activity was assessed using excisional wound healing method and anti-inflammatory activity was assessed using carageenan induced paw oedema model. Acute oral toxicity of the extract was determined by giving the extract at the rate of 2000 mg/kg by single oral dose. For testing the wound healing activity, the methanolic extracts of the plants were used at 5% and 10% in paraffin vehicle and the effect was compared with the standard drug boric acid. Anti-inflammatory activity was assessed by single oral dose at the rate of 250 mg/kg and 500 mg/kg of the extracts subsequent to carageenan, keeping diclofenac as positive control. It was found that both plant extract did not possess any significant toxicity after oral dosing. *H. colorata* showed excellent wound healing activity and *G. arborea* showed moderate activity. With respect to the anti-inflammatory activity, *G. arborea* was having better activity than *H. colorata* extract, though both were significantly less effective than diclofenac. Both the plant extract showed significant wound healing as well as anti-inflammatory activities.

Keywords

Gmelina arborea,
Hemigraphis colorata, Excisional wound healing, Carageenan induced paw oedema.

Article Info

Accepted:
26 June 2017
Available Online:
10 August 2017

Introduction

Wound is defined as disruption of cellular, anatomical, and functional continuity of a living tissue. It may be produced by physical, chemical, thermal, microbial, or immunological insult to the tissue. Optimum healing of a cutaneous wound requires a well-orchestrated integration of the complex biological and molecular events of cell migration and proliferation and of extracellular matrix deposition and

remodelling (Yusof, 2005). Inflammation is a complex biological response of vascular tissue to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain (Palladino *et al.*, 2003).

Large group of medicinal plants are used as traditional medicine, which have potential to cure various ailments. These medicinal plants which contain potential sources of phyto

compounds are used for many therapeutic purposes (Murugesan and Deviponnuswamy, 2014). Plants have the immense potential for the management and treatment of wounds. A large number of plants are used by tribal and folklore in many countries for the treatment of wounds and burns. These natural agents induce healing and regeneration of the lost tissue by multiple mechanisms. These phytomedicine are not only cheap and affordable but are also safe (Thakur *et al.*, 2011).

Gmelina arborea, (Beechwood), is a fast-growing medium to large deciduous tree that reaches 35 m in height and more than 3 m in diameter, occurring naturally throughout greater part of India at altitudes up to 1,500 meters. Also its presence is noted in Bangladesh, China, Pakistan, Myanmar, Nepal, Vietnam, Thailand, and Sri Lanka (Dvorak, 2004). It is found to possess *in vitro* antioxidant (Patil *et al.*, 2009), hepato protective (Sinha *et al.*, 2006), anti-diabetic (Kulkarni *et al.*, 2013), insecticidal (Vitrata and Tomentosicollis, 2005) and anti-bacterial activities (El-Mahmood *et al.*, 2010).

Hemigraphis colorata (Acanthaceae), an exotic plant adapted to India, is a versatile tropical low-creeping perennial herb that reaches a height of 15 to 30 cm. It prostrates and spreads with rooting stems when grown on ground. The leaf has metallic purple lustre on upper surface and a solid dark purple on ventral side. The leaves are opposite, ovate to cordate, serrate-crenate, about 2 to 8 cm long and 4 to 6 cm wide, bearing well-defined veins (Priya, 2012). This plant is found to have antibacterial (Anitha *et al.*, 2012; Jayaprakasan *et al.*, 2014), anti-elastase activity (Gangadharan *et al.*, 2013) and antioxidant (Akhil and Prabhu, 2013). Detailed pharmacognostical and phytochemical study of the plant was done by Saravanan *et al.*, (2010).

The above mentioned plants are used by tribal and folklore healers for the treatment of inflammation and wound in the Wayanad district of Kerala. Hence a study was conducted to systematically evaluate the wound healing activity and anti-inflammatory screening of this plant in appropriate rat models.

Materials and Methods

Adult healthy male wistar albino rats of 6 months of age weighing 200-250g were used for the study. Animals were maintained under uniform standard managemental condition at the institutional laboratory animal facility. Animals were given standard rat feed. Clean water was provided *ad lib*. The experiments were approved by the institutional animal ethics committee of the institute.

The plants *Gmelina arborea* and *Hemigraphis colorata* were collected locally and were identified by a botanist. The aerial parts were washed and dried in shade, pulverised and kept in air tight container at room temperature till extraction.

Preparation of methanolic extract

Pulverised plant was extracted with methanol in a soxhlet extraction unit at 70° C successively for five times. Methanol was removed by rotary vacuum evaporator (M/s Buchi, Switzerland) and dried till entire methanol was evaporated.

Acute oral toxicity of the extracts

Acute oral toxicity of the extracts were assessed according to OECD 425 guidelines at limit dose of 2000 mg/kg body weight give orally as a single dose for three animals. The animals were observed for any morbidity and mortality upto 14 days and at 15th day the animals were slaughtered humanely and the

organs were examined for any pathological changes.

Wound healing activity

Excisional wound healing method as described by Morton and Malone (1972) was used for the assessment. Animals were divided into six groups of six animals each based on the treatment done or the wounded area, as given below

Group I Control: - White soft paraffin

Group II Standard drug: 10% boric acid in white soft paraffin

Group III: Test extract *Gmelina arborea* 5% in white soft paraffin

Group IV: Test extract *Gmelina arborea* 10% in white soft paraffin

Group V: Test extract *Hemigraphis colorata* 5% in white soft paraffin

Group VI: Test extract *Hemigraphis colorata* 10% in white soft paraffin

After the animals were anaesthetized dorsal fur was shaved and a full thickness of the excision wound of 1 cm² was created along the markings using a surgical blade and pointed scissors. The entire wound was left open. The wound closure rate was assessed by tracing the wound on days all days till it healed, using transparency paper and a permanent marker. The wound areas recorded were traced to a graph paper and the area was measured. Number of days required for falling of eschar without any residual raw wound gave the period of epithelisation. The reduction in wound area on everyday was calculated using the formula % reduction in wound area on nth day = (initial wound area – wound area on nth day) X 100/ initial wound area.

Anti-inflammatory activity

Anti-inflammatory activity was measured using carrageenan induced paw oedema in rats (Morris, 2003). Animals were divided into six groups of six animals each, as given below

Group I Control: - Distilled water

Group II Standard drug - Diclofenac 30 mg/kg oral

Group III: Test extract - *Gmelina arborea* 250 mg/kg in water

Group IV: Test extract - *Gmelina arborea* 500 mg/kg in water

Group V: Test extract - *Hemigraphis colorata* 250 mg/kg in water

Group VI: Test extract - *Hemigraphis colorata* 500 mg/kg in water

Paw thickness was measured using vernier callipers. The basal paw thickness was measured before the administration of inflammatory agent. Carrageenan (M/s HiMedia, India) 1% w/v was dissolved in 0.9% normal saline solution and was given as sub-plantar injection using 25G needle to the right hind paw of each animal.

Paw volume was measured at 1, 2, 3 and 4 hours and the increase in paw volume was calculated by subtracting the basal paw thickness. The percentage increase in paw thickness was calculated as % increase = (Volume at nth hour- basal volume) X100/ Basal volume.

The results were analysed statistically using SPSS and Tukey's post-hoc tests for significance at 5% and the results are expressed as mean ± SEM.

Results and Discussion

Acute oral toxicity of the extracts

Upon administration of the extract at the dose rate of 2000 mg/kg, no deaths were observed in any of the extract group. This result indicates that there is no acute oral toxicity for the drug even at limit test dose of OECD 425 guideline. Hence this extract can be considered as practically non-toxic.

Wound healing activity

The results of reduction in wound area in excisional wound healing model are given in table 1. The result showed that both the extracts possess wound healing activity when compared to the control drug. Among them, *H. colorata* had better wound healing activity especially at the higher dose of 10% ointment as compared to even boric acid. From the day 4, the effect was better than the boric acid as given by the reduction of wound area by $58.12 \pm 3.76\%$ in boric acid treated group while the reduction in wound area was $59.15 \pm 2.12\%$. The complete wound healing was observed two days earlier (seventh day) in *H. colorata* 10% group and boric acid group, one day earlier (eighth day) in all other groups except the lower concentration of *G. arborea* 5% (98.12 ± 3.23). The general effect of *G. arborea* was less than that of *H. colorata* and boric acid, but was superior to that of control. On visual observation, all the treatment groups with plant extracts were having less visible signs of inflammation than the control as well as the boric acid groups.

Anti-inflammatory activity

The results of reduction in wound area in excisional wound healing model are given in table 2. From the result it is clear that both the extracts possessed significant anti-inflammatory activity than the control group,

the effect being more for *G. arborea* than *H. colorata*. But both the extracts were having lower effect than the standard drug diclofenac. The acute anti-inflammatory effect was less than that of diclofenac as indicated by a lower value of reduction in paw thickness of 10.23 ± 2.21 and 15.65 ± 2.76 respectively for *G. arborea* 250 mg/kg and 500 mg/kg and 7.56 ± 1.43 and 11.45 ± 2.09 respectively for *H. colorata* 250 mg/kg and 500 mg/kg, whereas it was 20.12 ± 1.45 for diclofenac. However, during the third hour of inflammation, the effect was comparatively better for both plant extracts. By fourth hour there was more than 50% resolution of inflammation in diclofenac group (79.11 ± 2.11), *G. arborea* 500mg/kg (62.16 ± 2.12) and *H. colorata* 500 mg/kg (54.33 ± 2.76).

In the present study it was found that both the plant extracts are having a significant wound healing as well as anti-inflammatory activity. *H. colorata* was having excellent wound healing activity which excels the standard drug boric acid. *G. arborea* also possess a good wound healing activity when compared to the negative group. Regarding the anti-inflammatory activity, *G. arborea* is better in its action compared to *H. colorata*, but the anti-inflammatory activity is significantly less for both the extracts when compared to the standard drug diclofenac.

Considering the fact that nearly 6 million people suffer from chronic wounds worldwide, wounds particularly chronic wounds are major concerns for the patient as well as for clinician as they seriously reduce their quality of life (Kumar *et al.*, 2007).

Wound healing is an intricate process initiated in response to an injury that restores the integrity and function of damaged tissues, which involves continuous cell-cell and cell-matrix interactions allowing to proceed in three overlapping phases *viz.* inflammation

(0–3days), cellular proliferation (3–12 days) and remodelling (3–6months) (Clark, 1996). Wound healing is complete when the disrupted surfaces are firmly knit by collagen and the process involves platelet aggregation

and blood clotting, formation of fibrin, alteration in the ground substances, angiogenesis and re-epithelialisation (Buffoni, 1993).

Table.1 Wound healing activity of methanolic extract of *G. arborea* and *H.colorata* in excisional Wound healing model (Mean % reduction in wound area ± SEM)

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
Control	5.98 ±1.98 ^a	11.98 ± 1.56 ^a	18.34 ± 1.95 ^a	28.17 ± 2.59 ^a	36.17 ± 1.09 ^a	59.93 ± 3.98 ^a	76.12 ± 2.96 ^a	89.55 ± 2.74 ^a	100 ± 0
Boric acid	14.91 ± 2.11 ^c	29.34 ± 1.06 ^c	43.34 ± 1.98 ^c	58.12 ± 3.76 ^c	69.12 ± 1.58 ^c	91.23 ± 3.22 ^b	100 ± 0 ^b	100 ± 0 ^b	100 ± 0
GA 5%	9.24 ± 1.23 ^b	16.25 ± 2.12 ^b	29.23 ± 2.12 ^b	35.12 ± 2.25 ^b	49.99 ± 2.23 ^b	75.12 ± 1.67 ^b	85.45 ± 2.29 ^b	98.12 ± 3.23 ^b	100 ± 0
GA10%	12.98 ± 1.03 ^c	19.98 ± 1.15 ^b	35.34 ± 1.33 ^c	43.17 ± 1.85 ^c	56.17 ± 3.22 ^b	89.93 ± 2.65 ^b	96.12 ± 3.12 ^b	100 ± 0 ^b	100 ± 0
HC 5%	11.34 ± 0.98 ^{bc}	20.11 ± 1.76 ^b	34.94 ± 1.15 ^c	42.47 ± 3.11 ^c	53.97 ± 1.03 ^b	82.15 ± 1.67 ^b	98.12 ± 1.87 ^b	100 ± 0 ^b	100 ± 0
HC 10%	13.45 ± 2.12 ^c	28.12 ± 1.04 ^c	42.65 ± 0.98 ^c	59.15 ± 2.12 ^c	72.12 ± 1.26 ^c	96.12 ± 3.22 ^b	100 ± 0	100 ± 0 ^b	100 ± 0

One way ANOVA with Tukey’s post hoc analysis. Values with different superscripts vary significantly

Table.2 Anti-inflammatory activity of methanolic extract of *G. arborea* and *H.colorata* in Carageenan induced paw oedema (Mean % reduction in inflammation ± SEM)

	1 hr	2 hrs	3 hrs	4 hrs
Diclofenac	20.12 ± 1.45 ^c	42.34 ± 2.21 ^c	63.55 ± 1.98 ^c	79.11 ± 2.11 ^c
GA 250 kg/kg	10.23 ± 2.21 ^b	23.11 ± 1.12 ^{ab}	34.77 ± 1.22 ^a	49.22 ± 1.19 ^a
GA 500mg/kg	15.65 ± 2.76 ^b	29.45 ± 2.77 ^b	51.44 ± 2.44 ^b	62.16 ± 2.12 ^b
HC 250 mg/kg	7.56 ± 1.43 ^a	17.54 ± 1.87 ^a	35.11 ± 2.01 ^a	43.12 ± 3.15 ^a
HC 500 mg/kg	11.45 ± 2.09 ^b	25.58 ± 2.22 ^b	45.22 ± 3.11 ^{ab}	54.33 ± 2.76 ^a

One way ANOVA with Tukey’s post hoc analysis. Values with different superscripts vary significantly

One of the major pathology of wound is the inflammation associated with it, which is the major cause of irritation in the wound.

Inflammation results in a coordinated influx of neutrophils at the site of wound and through their characteristic “respiratory burst” activity start producing free radicals (Baboir, 1978). Hence, an agent which is simultaneously having the wound healing and anti-inflammatory effect will be an excellent

remedy in the management of wounds. The respiratory burst at the local area cause an elevated reactive oxygen species and reactive nitrogen species at the local site of wound, which will in excess can cause severe oxidative damage to the skin itself, including both cellular and extra cellular elements.

To accelerate wound healing and diminish the risk of tissue damage induced inflammation, exogenous substances, it is better to apply

substances which could interfere with wound-associated ROS (RNS)-mediated processes (Korkina *et al.*, 2007).

The concept of developing drugs from herbs in indigenous medical system is much older, while in some cases direct links between a local and biomedical use exists, in some other cases the relationship is more complex (Heinrich and Gibbons, 2001).

In the present study, it is established that both the compounds were having wound healing as well as anti-inflammatory activity. Previously, Patil *et al.*, (2009) reported the antioxidant effect of *G. arborea* and Annan and Houghton (2008) reported the anti-oxidant activity of *H. colorata*.

Since the plant extracts are having all the qualities required for a wound healing and anti-inflammatory drug, it is safe to presume that this indigenous knowledge is a valid one and it can be safely used, considering its lack of toxicity for mammalian systems.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this research paper.

Acknowledgements

Financial supports from Indian Council of Agricultural Research (ICAR) through research projects (NAIP/C2066, NFBSFARA/BSA-4004/2013-14, NASF/ABA-6015/2016-17, No. 7(2)/- 2011- EPD) and National Bank for Agriculture and Rural Development G.O.(Rt)No.100/12/ AD RIDF XVI KERALA) are thankfully acknowledged.

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How to cite this article:

Bhagyalakshmi Chengattu Prakashbabu, Deepthi Vijay, Saju George, Sameer Kodiyil, Suresh Narayanan Nair, Ajithkumar Karaparambil Gopalan, Sanis Juliet and Reghu Ravindran. 2017. Wound Healing and Anti-Inflammatory Activity of Methanolic Extract of *Gmelina arborea* and *Hemigraphis colorata* in Rats. *Int.J.Curr.Microbiol.App.Sci*. 6(8): 3116-3122.
doi: <https://doi.org/10.20546/ijcmas.2017.608.373>