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Formulation and Evaluation of *Andrographis paniculata* Topical gel for Wound healing activity

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ABSTRACT:

The present study aimed to formulate the topical gel of *Andrographis paniculata* (A.paniculata) extract and to evaluate the gel for its physical properties, *in-vitro* permeation, and wound healing properties in rats. Materials and Methods: Topical gels were prepared using two polymers namely Carbopol and Hydroxypropyl Methyl Cellulose (1-1.5%). The gels were evaluated for their viscosity, spreadability, content uniformity. *In-vitro* diffusion study was conducted for topical gels using Franz diffusion cell for the biomarker compound β-sitosterol. Results and Discussion: It was observed that with an increase in the polymer percentage, there was a proportional increase in the viscosity and reduced spreadability. Similarly, the rate of diffusion of the drug from the gels was also reduced with an increase in the polymer concentration. All the prepared formulations demonstrated content uniformity and moderate to good spreadability. Treatment with Topical gel decreased wound contraction time. The fall of eschar left no raw wound behind (0.397±0.675). Percentage wound contraction (96.22) in 14-15 days, the results of the wound healing activity were significantly better than the control group. Conclusions: It can be concluded that *A. paniculata* loaded Topical gels is a viable option for the treatment of wounds.

Key words: Topical gel*A.paniculata,* SEM, Excision wound.

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Introduction

Andrographis paniculata (AP) is a common medicinal plant used all over the world. It is a member of the Acanthaceae family. In Bangladesh, China, Hong Kong, India, Pakistan, the Philippines, Malaysia, Indonesia, and Thailand, AP is used as a traditional herbal medicine. (S. Akbar 2011, M.

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H. Kabir 2014) and it is an ethno-medicinal used for the treatment of snakebite, bug bite, diabetes, dysentery, fever, and malaria (I. H. Burkill1996).the plant contain flavonoids, Phytosterols, diterpenoids, Bitter glycosides, Phenols and Xanthones (S. Akbar 2011, M. H. Kabir 2014, K. Jarukamjorn and N. Nemoto 2008, Damu AG1998, Koteswara Rao Y2004, C. Xu,2012, C. Boopathi2000)Plant have reported serval activities such as Herbal extracts of *A. paniculata* are useful as an anti-inflammatory (Sheeja, Shihab, & Kuttan, 2006), antioxidant (Tripathi & Kamat, 2007), antiviral (Calabrese et al., 2000), anticancer (Ajaya et al., 2004), antimicrobial (Singha, Roy, & Dey, 2003), antimalarial (Siti Najila et al., 2002), and are hepatoprotective (Trivedi, Rawal, & Patel, 2007) agents. It had also shown immunostimulatory (See, Mason & Roshan, 2002), phagocytotic (Matsuda et al., 1994), anti-diabetic (Reyes et al., 2006), and hypotensive (Zhang & Tan,1996) activities. However, there is no information on the ability of A. paniculata extracts to heal wounds. Thus, the aim of this study was to determine if ethanolic extracts of *A. paniculata*could help heal wounds.in rats.

Materials and methods

Plant Materials

The Ethanolic extract of *A.paniculata* batch no-210316/01 is gift sample obtained from Himalaya drugs, Bangalore, India.

Description and Solubility

Ethanolic extract *A. paniculata* (EAP) as describe the organoleptic properties and solubility with polar solvents.

Herbal extract-excipient compatibility study

Extract -excipient compatibility study was carried out to investigate any possible interaction between *A. paniculata* and other excipients used in the formulation of the Topical gel, the samples were analyzed byFTIR spectroscopy

FTIR studies of extract of *A. paniculata,*β-sitosterol,its physical mixture was done by using the FTIR spectrometer (IR Affinity-1S). The samples were grounded and mixed thoroughly with KBr and the spectrum was noted in the range of 4000 – 400 cm^{-1}.

PREPARATION OF CALIBRATION CURVE OF β-SITOSTEROL

10 mg of β-sitosterol was taken in a 10ml standard flask and dissolved in Phosphate buffer PH 6.8. The volume of the stock solution was made up to 10ml with Phosphate buffer PH 6.8. From the above stock solution different aliquots of 0.5, 1, 1.5, 2, 3, 4, and 5ml were transferred to 10ml volumetric flask, volume was adjusted with Phosphate buffer PH 6.8., which gave a concentration

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of 50, 100, 150, 200, 300, 400 and 500µg/ml of the final standard. The standard curve was plotted by taking absorbance of secondary stock solutions in UV double beam spectrophotometer at 208 nm for β-sitosterol.

Preparation of Ethanolic Extract of*A.Paniculata* Topical **Gels**

Topical Gels of ethanolic extract of *A. paniculata*were prepared as described below

1. Weigh 1gms of extract *A. paniculata*.

2. The specified amount of Carbopol 934 andHPMC, carbopol 934powder was slowly added to ultrapure water and kept for 12 hours for the polymer to swell.

3. Ethanolic extract of AP was dissolved and sodium benzoate was added to it, this mixture was incorporated to the above mixture and was subjected to After complete addition, the mixture was stirred continuously at 800rpm until homogeneous gels were obtained. These formulations were then placed in wide-mouthed bottles for stability testing, with all samples equilibrated at room temperature. There were six different formulations. as shown in (Table**1**)(F1, F2, F3,F4,F5 and F6) were prepared using a varying concentration of Carbopol 934P and HPMC (Dave *et al.*, 2010; Helal *et al.*, 2012).

NAME OF THE INGREDIENTS	**Quantities in w/w %(100 gm)**					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
EAP	10	10	10	10	10	10
PEG 400 (V/V)	5	5	5	5	5	5
Carbapol 934 (% W/V)	1	1.5	2	-	-	-
HPMC	-	-	-	1	1.5	2
Sodium benzoate (W/W)	1	1	1	1	1	1

Table1:Composition of Topicalgelformulations

Characterization of EAP loaded TopicalGel

Zeta potential of EAP Topical Gel

Zeta potential of Topical gel formulations were measured by dynamic light scattering (DLS) technique (Malvern Zetasizer, Malvern Instrument, UK). Samples were dispersed in distilled water (3:25) before measurement.

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Morphology of EAP TopicalGel

Topical gel morphology was observed using scanning electron microscopy (SEM) (Microscope Tecnai 200 kV D2360, USA). A drop of the Topical gel that had been dispersed by water was placed onto the carbon-coated copper grid and dried at room temperature, leaving a thin film. The film was colored using phosphotungstic acid solution and imaged.

Evaluation of EAP Topical Gel
Spreading diameter

By calculating the spreading diameter of 1 g of gel between two horizontal plates (20 cm 20 cm), the spreadability of the gel formulation was determined after one minute. On the upper plate, the normal weight was 125 g. (Misal J, Dixit G, Gulkari V2012)

Viscosity and pH measurement

Viscosity of Topical gelformulations was measured using Brookfield viscometer (Model No DV-III ULTRA) using spindle no 06 at 100 rpm, and pH measurements of the formulations were done using digital pH meter (RI-152-R).

***In vitro* diffusion studies ofEAP TopicalGel**

In-vitro diffusion study was performed for topical gel dispersion (F1, F2, F3, F4, F5, and F6), Topical gel formulation using dialysis membrane (Hi media). Diffusion membrane was placed in Phosphate buffer solution (PBS) 7.4 for 6 h to attain saturation before starting permeation study and then mounted between the donor and receptor compartment of the Franz diffusion cell (fabricated with glass, the surface area available for diffusion was 2.54 cm2). The release rate of EAP was analyzed by placing the required sample in the donor cell compartment. To prevent contamination and evaporation, the donor compartment was covered with parafilm. The receptor chamber was filled with PBS 7.4 and was maintained at 37°C with continuous stirring. 1 ml aliquot of receptor phase solution was withdrawn at half an hour from the commencement of diffusion studies, followed by every hour till approximately 80% of the drug was released, the same volume of fresh medium was added back into the receptor compartment to maintain the sink conditions. The quantification was done using a UV spectrophotometer (Shimadzu Model No. 1800) at 208 nm. The cumulative amount of drug diffused versus the time graph was plotted. (Dave.*et al.*, 2010; Hamed R*et al.*, 2016, Lonni AA *et al.*, 2010, Patel MR AA *et al.*, 2016)

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Stability study of EAP TopicalGel

Stability evaluation of Topical gel was performed by storing the gel at high (40°±2°C), room (25° ±2°C) and low (7°± 2°C) temperatures. During 12-weeks, organoleptic changes, pH in the EAP Topical gel were evaluated.

Pharmacological study on wound healing activity

Experimental animalsprocured

Male adult Wistar rats of 9 to 11-weeks, weighing 180–250g were procured from Mahaveera enterprises, Hyderabad. Animals were housed in standard laboratory conditions at 25°c with 12 hlight-dark cycle with free access to chow and water *ad libitum*. The research protocol was approved by (IAEC/1657/CMRCP/T2/PhD-16/84)

Excision wound model

Group I served as Control
Group II served as Excision wound
Group III served as EAP Topical gel + Excision wound
Group IV served as AVOMEB ointment +Excision wound

Male Albino rats 180-250 gm were taken for studies, the rats were anesthetized before and during the infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using Anesthetic Ether. A wound of 500 sq. mm on the dorsal thoracic region was made. Animals were applying the gel daily and closely observed for any infection and those which showed signs of infection were separated and excluded from the study and replaced. The animals were observed for wound closure at 0, 4^{th}, 6^{th} ,8^{th}, $12^{th}$and 15^{th} day and for period of epithelialization. (Roshan, S *et.al* 2008, Sultana, Z *et.al*,2015, Nasir,M.*et.al*2016)

Measurement of wound area

The changes in the wound area after the application of the gel were recorded on 2^{nd}, 4^{th}, 6^{th},8^{th},

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12th and 15th day. The wound size was also assessed every day with a scale, and the wound area was noted.

Wound contraction was calculated as the percentage of the reduction in wound area. (Nayak BS*et.al* 2009)

$$\text{Percentage of wound contraction} = \frac{\text{(Initial wound area – Specific day wound area)}}{\text{Initial wound area}} \times 100$$

STATISTICAL ANALYSIS

Data are expressed as mean ± standard error of mean. Differences in the *in vitro* release profile of prepared formulations were tested for significance using independent *t*-test using SPSS-17.0. Difference was considered significant when$P < 0.05$. Graphs were prepared using GraphPad Prism 8 (Graph Pad Software, Inc). ***p<0.001, **p<0.01 and *p<0.05.

RESULTS AND DISCUSSION

Physical properties of the gel

The prepared topical gels of AP demonstrated moderate to good spreadability and from the results it was evident that the gel spreadability was dependent on the polymer concentration and was reduced with an increment in the polymer concentration. The viscosity of the gels though increased with the increase in the polymer concentration, however, the gels remain easily spreadable. Polymer concentration affected the diffusion rate of the drug and the release was sustained with an increase in polymer concentration. Carbopol gels demonstrated higher viscosity compared to the corresponding HPMC concentrations.

The IR spectra for drug excipient compatibility study showed major peaks at 3328.77 cm^{-1},3298.17 cm^{-1}, 2983.88 cm^{-1},2833.98 cm^{-1},1803.51 cm^{-1}, 1640.15 cm^{-1},1653.29 cm^{-1}, 1044.13 cm^{-1}, 2934.07 cm^{-1}, 2867.37 cm^{-1},1709.71 cm^{-1}and1462.09 cm^{-1}in pure extract the corresponding peaks were also obtained in the extract excipient mixture with slight shifting. It is evident from the data that the characteristics peaks of extract were not affected in the presence of Carbopol and HPMC, implying that extract and excipient are compatible with each other [Figures1,2 and 3].

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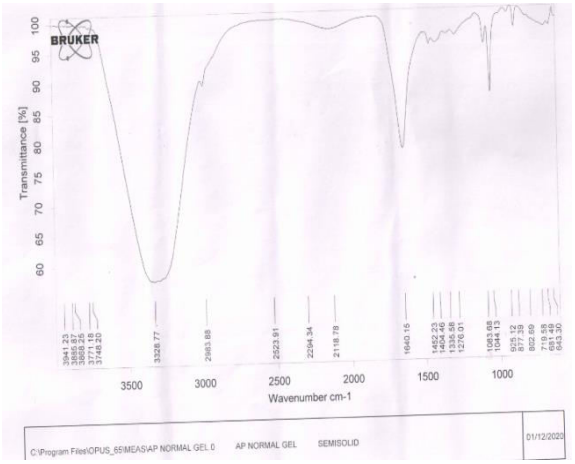


Figure 1 : IR spectra of*A. paniculata* Topical gel

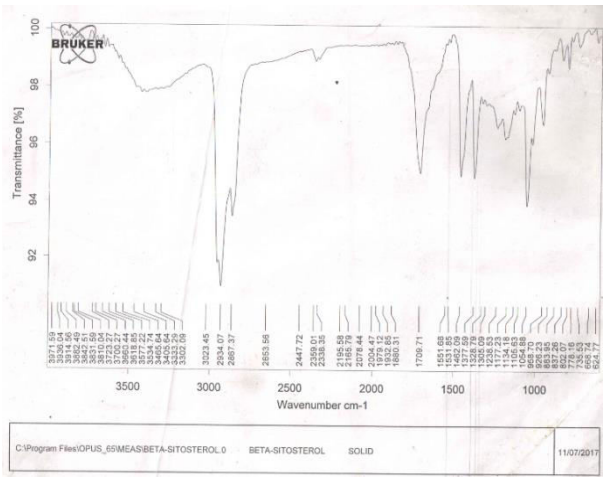


Figure 2: IR spectra of β-sitosterol

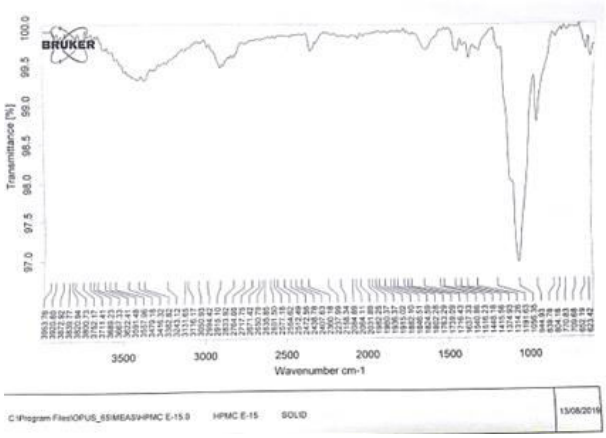
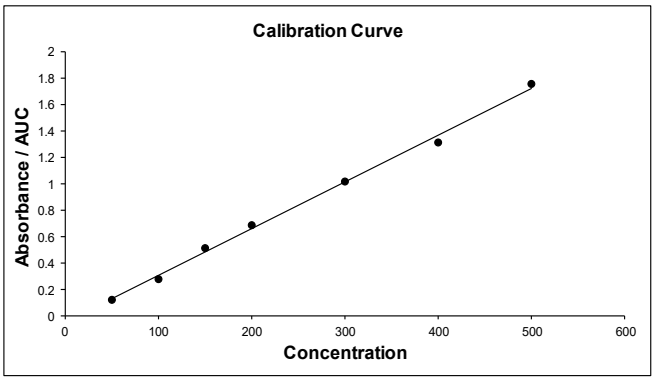


Figure 3: IR spectra of HPMC



Graph1: Calibration curve of β-sitosterol

CALIBRATION CURVE OF β-SITOSTEROL

Graph1: Calibration curve of β-sitosterol

Characterization of EAP loaded TopicalGel

The preliminary characterization of EAP topical gel (prior to sonication) was done by using an optical microscope.

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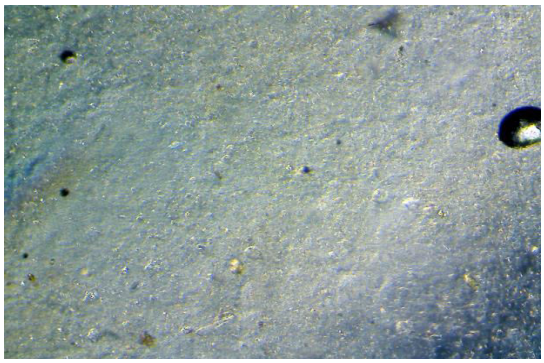

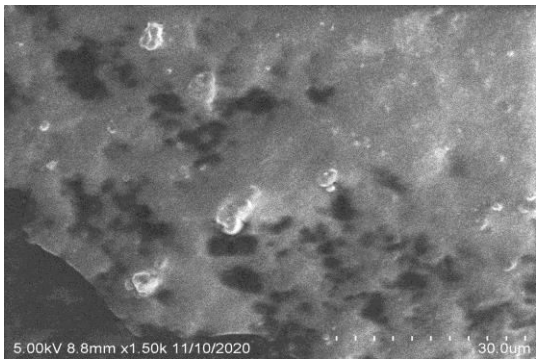

Figure 4: optical microscope image of AEP Topical gel Figure 5 : SEM image of AEP Topical gel

Overall, performance of transdermal drug delivery system is generally governed by morphology. Scanning electron micrograph of Topical gel[Figure 4]. Phase contrast microscopy also showed the surface morphology of Topical gel [Figure 5] All the images depict smooth surface.

ZP is an important parameter that affects stability. All the ethosomal formulation were found to have negative ZP (−50.0 to −62.4 mV) due to the net charge of the lipid composition in the formulation. The negative ZP is responsible for enhanced percutaneous permeation of drug.

Measurement Type	:	Zeta Potential
Sample Name	:	AP Normal Gel-Zeta
Temperature of the holder	:	25.2 deg. C
Viscosity of the dispersion medium	:	0.892 mPa.s
Conductivity	:	0.353 mS/cm
Electrode Voltage	:	3.3 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-50.0 mV	-0.000389 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	:	-50.0 mV
Electrophoretic Mobility mean	:	-0.000389 cm²/Vs

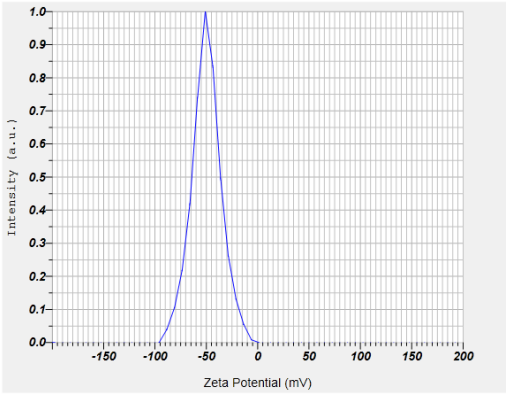

Figure 6: zeta potential of *A. paniculata* Topical gel

Evaluation of EAP TopicalGel

The prepared gels were evaluated for physical appearance, pH, spreadability, viscosity, and drug content. Gels were found to be smooth, homogenous, yellowish white in color, pH lying in the normal skin pH range, easily spreadable, and viscosity ranging between 4500 and 4800 cps

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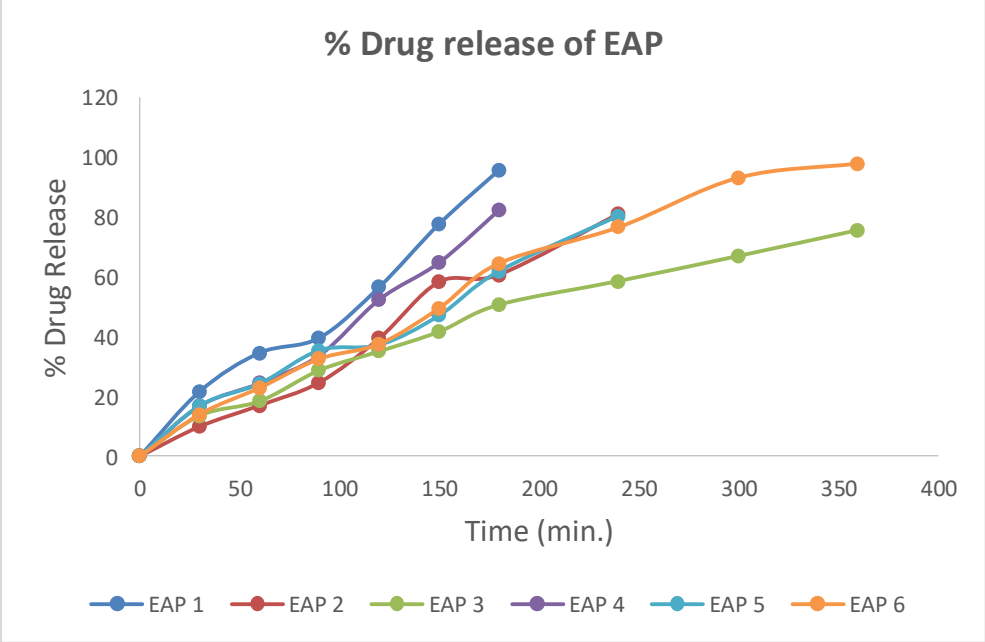
Formulation	pH	Viscosity (CPS)	Spreadability (g.cm./sec.)	Grittiness	Drug Content
EAP1	6.7	35410	34.10	No	98.20
EAP2	6.8	40240	32.35		97.35
EAP3	6.9	43458	30.47		96.85
EAP4	6.9	31022	32.32		98.70
EAP5	6.9	36540	31.75		97.85
EAP6	6.9	38878	29.80		97.10

Table2: Evaluation of EAP Topical gel formulations



Graph:2 *in vitro* kinetic release of EAP topical gel

Topical Gel on excision wound:

The results of Herbal Extract of A. paniculata loaded Topical Gel on excision wound. the wound healing is recorded in (table) The wound healing control group, wound contraction rate and % of wound contraction are observed in days 20-23(0.510±0.214) and % wound contraction 82.61 0n 20th day. On treatment with Topical gel decreased wound contraction observed to monitor the fall of eschar leaving no raw wound behind (0.268±0.5745) and % wound contraction (97.70) in 13-14days, the results are comparable with that of showing better healing compared to control. The

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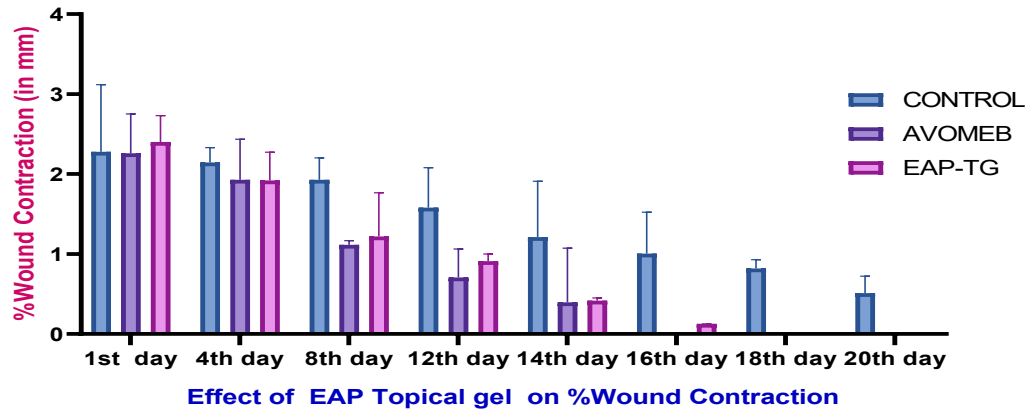
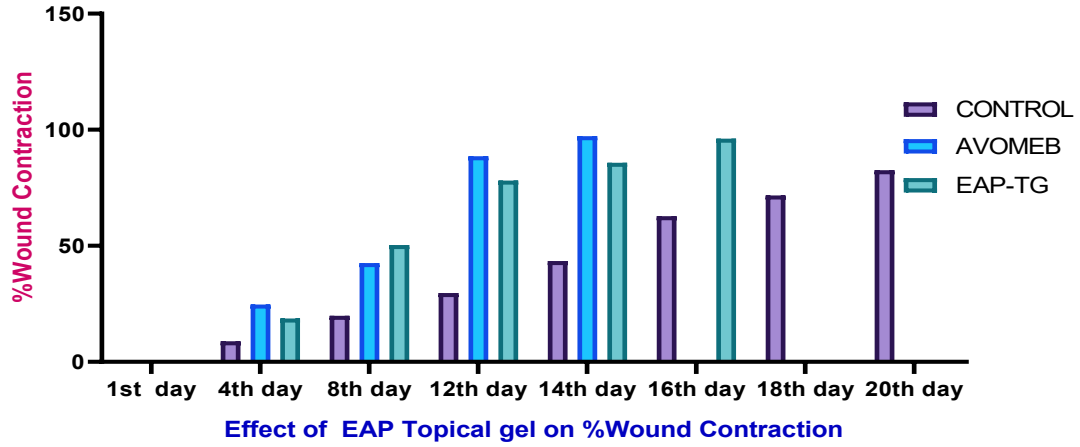
treatment Standard Avomeb was day 14-15 are observed to monitor the fall of eschar leaving no raw wound behind, the results obtained indicate enhancement of wound contraction rate (0.161±0.890) and %97.20 wound contraction and increased epithelization followed by fall of eschar, with the excision wound model.

% Wound Contraction with Topical Gel								
DAY	**1st day**	**4th day**	**8th day**	**12th day**	**14th day**	**16th day**	**18th day**	**20th day**
CONTROL	2.279±0.83 00.00	2.146±1.18 8.85	1.928 ±1.67 19.83	1.581±1.499 29.67	1.211±1.70 43.46	1.006±0.51 62.81	0.819±0.11 71.66	0.510±0.214 82.61
EAP-TG	2.259±1.49 00.00	1.927±1.50 24.69	1.114±1.054 42.61**	0.708±1.354 88.61***	0.397±0.675 97.22***	-	-	-
AVOMEB	2.40±1.330 00.00	1.919±1.35 18.70	1.219±1.546 50.35	0.912±0.086 78.13***	0.416±0.031 85.79***	0.126±0.00 98.20***	-	-

Table 3: wound healing activity of EAP gel



Graph 3: Effect of EAP Topical gel on wound contraction in excision wound



Graph 4: Effect of EAP Topical gel on %wound contraction in excision wound

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Antioxidant properties have been identified for *A.paniculata* (Trivedi & Rawal, 2001). Antioxidants have been shown to help with wound healing and tissue protection against oxidative injury (Martin, 1996). Phytochemical analysis of the A. paniculata extract revealed the existence of flavonoids and diterpenoids, andrographolides, - sitosterol, stigmasterols, 4-ketopinoresinol, and - Amyrin, among other bioactive molecules (Koteswara, Vimalamma, Venkata Rao, & Tzeng, 2004). The wound-healing activity may be attributed to these phytochemical constituents. Flavonoids are known to aid wound healing because of their antimicrobial properties, which tend to be responsible for wound contraction and increased epithelialization rates.(Tsuchiya et al., 1996).

CONCLUSION

The prepared Topical gels incorporated with *A.paniculata* extract have shown enhanced permeation profile as compared to the Avomeb, conventional formulation. We conclude that transdermal delivery of *A.paniculata* extract through topical system may be a better approach for wound healing. However, further biological and clinical research have to be carried out to explore the therapeutic potential of this extract for treating wound healing other modules and anti-bacterial properties which will be helpful in the development of safe and efficacious herbal formulations.

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CONFLICT OF INTEREST

We have no conflict of interest to declare.

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