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DESIGN AND CHARACTERIZATION OF *ALLAMANDA CATHARTICA* BASED OINTMENT FOR ITS WOUND HEALING PROPERTY

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ABSTRACT

The objective of the present investigation was to study the wound healing activity of petroleum ether extract of *Allamanda cathartica* Leaves ointments in animal model. *Allamanda cathartica* belongs to the family *Apocyanaceae.* The prepared extract was subjected to phytochemical screening to determine the presence of various phytoconstituents. Petroleum ether extract of *Allamanda cathartica* (AC) was subjected to formulation of Ointments consisting various bases viz., oleaginous base, Absorption base, Hydrophilic base and PEG bases by traditional incorporation method. Owing to all the results of physical parameters characterization, Ointment formulations of AC extract containing Hydrophilic base (OF3, OF7) and PEG base (OF4, OF8) were subjected to *in vitro* drug diffusion studies. OF3 and OF4 formulations comprising low concentration (5%) of Hydrophilic and PEG bases have shown good dissolution characters by releasing 90.76% and 85.43% active principle. There was no evidence of phase separation, development of disagreeable odour, change in colour, consistency and no change in parameters like pH, Viscosity, Spreadability of the all the products during stability study for three months. In the *in vivo* study the topical application of ACHB and ACPEG of petroleum ether extract ointments on the infected wound of the rats caused a significant ($P<0.05$) and faster rate of wound closure and reduced the epithilization period.

INTRODUCTION

A wound is defined as a disruption in the continuity of the epithelial lining of the skin or mucosa resulting from physical or thermal damage. According to the duration and nature of healing process, the wound is categorized as acute and chronic [1& 2]. An acute wound is an injury to the skin that occurs suddenly due to accident or surgical injury. It heals at a predictable and expected time frame usually within 8-12 weeks depending on the size, depth and the extent of damage in the epidermis and dermis layer of the skin [3& 4]. Chronic wounds on the other hand fail to progress through the normal stages of healing and cannot be repaired in an orderly and timely manner [5&6]. Chronic wounds generally results from decubitus ulcer, leg ulcer and burns. Our plant *Allamanda cathartica* belongs to the family Apocyanaceae. It is

native to Central America and Brazil and also grown in Indian gardens .*Allamanda cathartica* is a woody, evergreen shrub grows up to 3 meters in height. The leathery leaves are lanceolate, acute or acuminate, and may either be opposite or in whorls of three or four. The yellow, trumpet-shaped flowers are 5.2 - 6.5 centimeters in diameter; cultivated forms tend towards larger blooms which may also be white, purple, pink or orange in color and are sterile. Reproduction is mainly through stem cuttings. The useful parts are Bark, Leaves & Flowers. All parts of the plant contain allamandin, a toxic iridoid lactone. Leaves and stems yield ursolic acid, β-amyrin and β-sitosterol. plumericin and isoplumericin from stem and root-bark, also from leaves and roots, besides plumieride and long chain esters. The root contains antileukaemic iridoid lactone, allamandin and two other iridoids, allamandicin and allamdin. The stems and leaves contain beta amyrin, beta-sitosterol and ursolic acid. Petals gave flavonoids—kaempferol and quercetin. Some of the studies are revealed that *Allamanda cathartica* is having purgative effect, wound healing, reversible antifertility effect, cytotoxic, antimicrobial and anti-inflammatory activities.

MATERIALS AND METHODS

Collection and Authentication of the plant material: The *Allamanda cathartica* plant material was collected and authenticated from Dr. K. Madhava chetty, Assistant Professor, S.V University, Tirupati. Leaves of *Allamanda cathartica* were used for the study.

Preparation of Leaves Powder and Extraction: 250gms of plant material (leaves) was ground to course powder. The procedure recommended in Indian Pharmacopoeia (Anonymous, 1966; 1985; 1996). All chemicals and solvents used for different studies were of analytical reagent / spectroscopy grade. The powdered leaf was extracted with petroleum ether by using soxhlet extraction method [7].

Qualitative phytochemical screening: The petroleum ether extract of *Allamanda cathartica* was subjected to qualitative phytochemical screening to detect presence or absence of various phytoconstituents using standard methods [8].

Preparation of *Allamanda catahartica* extracts ointments using various bases

Petroleum ether extract of *Allamanda cathartica* was subjected to formulation of Ointments consisting various bases viz., oleaginous base, Absorption base, Hydrophilic base and PEG bases by traditional incorporation method. The composition of various respective ointment bases are listed from Table 1 to 4. OF1, OF2, OF3 and OF4 ointment formulations of *Allamanda cathartica* consists of 5% concentration of respective ointment bases, whereas OF5, OF6, OF7 and OF8 ointment formulations of *Allamanda cathartica* extract consists of 10% concentration bases. Compositions of Ointment formulations OF1 to OF8 were given in Tables 5& 6. All the four types of ointments were prepared by incorporation method in which all the components were mixed until a uniform preparation was obtained. 1 gm of AC leaf extract and 9 gms of respective ointment bases were weighed and mixed on an ointment slab using spatula by geometric dilution technique. Finally required quantities of preservatives were added.

Evaluation of Ointments [9, 10]. All the formulated AC extract ointments were subjected to various characterizations for assessment of different physico chemical parameters.

Spreadability Determination: Spreadability of formulation was determined by an apparatus which was fabricated in laboratory and used for the study. The ointment was placed between two glass slides and a weight of 1000g was placed on the slide for 5 minutes to compress the sample to a uniform

thickness. Weight of 70g was added to weighing pan. Now the time in seconds required to move the slides was taken as the measure of Spreadability. The following formula is required for the calculation of Spreadability.

S = m.l/t, Where S = Spreadability, m = weight tied to upper slide, l = length of slide in cm and t = time taken to separate two slides.

pH determination: The pH of formulated ointment was determined using digital pH meter available in college.

Viscosity determination: 100gm of each formulation was weighed and transferred to a beaker and viscosity was determined by using Brookfield dialed viscometer (USA), model RV, using spindle no. 7 at 50 RPM .

Extrudability: A simple method was adopted for this study. The formulations were filled in the collapsible tubes after the ointments were set in HDPE containers. The extrudability of the different ointment formulations was determined in terms of weight in grams required to extrude a 0.5cm ribbon of ointment in 10 second.

Stability study determination: The formulations were subjected to accelerated stability testing study according to ICH guidelines for finished pharmaceutical products. The formulations were placed at 40° C ± 2°C at 75 % ±5 % RH for a period of 3 months and sample were evaluated for various parameters like pH, viscosity, Spreadability and organoleptic property at an interval of 15 days.

Thermal Cycling: Most of the drug product, especially semisolid dosage form like ointment, creams, suspension and emulsions, lotions and suppositories may be adversely affected by variation in extreme temperature fluctuations. These types of drug products should be tested under cycling temperature condition to avoid this problem during storage and shipping. The prepared formulation was packed in container and placed in following storage conditions: 5° – 40°C in 24 h cycle for two weeks. The sample was tested for its organoleptic and physicochemical properties.

Drug content: Each formulation (1gm) was accurately weighed and transferred to 100ml volumetric flask to which about 70ml of methanol was added. After shaking, the volume was made up to 100ml with. The content was filtered through a suitable filter. 1ml filtrate was taken, diluted and the drug content (extract) was estimated by using UV/Visible spectrophotometer, (SL-159-Shimadzu- 1700, SI-164 Double Beam) at 270 nm.

Drug release studies by Franz Diffusion Cell: The *in vitro* diffusion studies of the ointments were performed by using dialysis membrane (Sigma Inc. MO, USA; dry, unwashed, pre-cut and open ended; fiat width: 35 mm; inflated diameter, 21mm; Length: 30mm). The membrane soaked in phosphate buffer pH 6.4 for 6-8 h was clamped carefully to one end of the hollow glass tube of dialysis cell (2.3 cm diameter, 4.16 cm2 area). 100ml of phosphate buffer was taken in a beaker, which was used as receptor compartment for the study. 1gm of each formulations both the ointments and gels were spreaded uniformly on the membrane. The donor compartment was kept in contact with the receptor compartment and the temperature was maintained at 37±0.5 °C. The solutions on the receptor side were stirred by externally driven Teflon-coated magnetic bars. At pre-determined time intervals, 5ml of solution from the receptor compartment was pipette out and immediately replaced with 5ml fresh phosphate buffer solution. The drug concentration of the receptor fluid was determined spectrophotometrically at 270nm against appropriate blank. The obtained results were subjected to drug release kinetic studies like zero order, first order, higuchi equation and erosion equation and peppas-korsemeyer equation.

EXPERIMENTAL ANIMALS: Albino wistar rats weighing 150 – 200gm was used throughout the experiment. The

animals were procured from Raghavendra Enterprises, Bangalore. Before imitation of experiment; the rats were acclimatized for a period of seven days. Standard environmental conditions such as temperature (26°C) relative humidity (45 – 55%) and 12 hrs dark / light cycle were maintained in the quarantine. All the animals were fed with the rodent pellet diet V.R.K Nutritional solutions, Pune, and water was allowed ad libitum under strict hygienic conditions. Ethical clearance for performing the experiments on animals was obtained from institutional animal ethics committee (IAEC). (REF.No 1423/Re/S/11/CPCSEA).

***IN VIVO* WOUND HEALING ACTIVITY ASSESSMENT [11]:** *in vivo* studies of the optimized topical ointment formulations were carried out using rats as the animal model to evaluate the wound healing activity of Petroleum ether extract of *Allamanda catahartica* by excision wound model. For this model Wister Albino rats (150-200gms) were selected and made into four groups of 6 animals each for the experiment. In which Group-I acts as control and receives only simple ointment base, Group-II acts as standard which receives Gentamycin ointment, Group-III receives 5%w/w Hydrophilic Base and Group-IV receives 5%w/w PEG Base. The animals were housed in the experimental room which was maintained as per IAEC guide lines. The experimental animals were anaesthetize with lignocaine 2% injections, over the local selected region. The rats were depilated over the region excision wound was infected by cutting a way of 25mm square thickness of skin from the predetermine area, the wound was left and rest to the open environment then the drugs reference standard (Gentamycin ointment) control (simple ointment base B.P) and *Allamanda cathartica* extract and different ointments prepared were applied till the wound was healed.

$$\% \text{ Protection} = 100 - \frac{\text{Final}}{\text{Initial}} \times 100$$

Model for Wound healing activity by In vivo studies:

Wound healing activity in Wistar Albino Rats. Excision wound model

a) Percentage of wound contraction

b) Period of epithelisation

RESULTS

Percentage yield: Plant part used: Leaves. Quantity of crude drug used: 250 g . Quantity of petroleum ether extract extract obtained: 9 g. Percentage Yield: 3.6% w/w

Preliminary phytochemical screening of crude extract: Petroleum ether extract of *Allamanda cathartica* leaves was subjected to preliminary qualitative phytochemical tests and inferred that the Glycosides, Alkaloids, Steroids, Triterpinoids and Amino acids were detected and where as Carbohydrates, Fixed oils, Tannins, Flavonoids, saponins, Gums and mucilages were not detected. The results are listed in Table No .7

Evaluation of Ointments: Various Ointment formulations of *Allamanda cathartica* Pet. Ether extracts (OF1 to OF8) were subjected to critical evaluation of some essential physical parameters viz., pH, spredability, viscosity, Extrudability, Thermal cycling and Drug content. OF3, OF4, OF7 and OF8 ointment formulations of *Allamanda cathartica* extract has shown pH values closer to that of optimal skin pH of 7, whereas other ointment formulations have shown much deviation from that of the required skin pH. Ointment formulations containing Hydrophilic and PEG bases (OF3, OF4, OF7 and OF8) have exhibited excellent Spreadability characters compared to Oleaginous and Absorption bases (OF1, OF2, OF5 and OF6). Ointment formulations containing Hydrophilic and PEG bases (OF3, OF4, OF7 and OF8) have shown high consistency in their behavior exhibiting high centipoises of viscosity compared to Oleaginous and Absorption bases (OF1, OF2, OF5 and OF6). OF7 formulation consisting Hydrophilic base at 10%

concentration was found to have extruded very conveniently compared to ointment formulations containing low concentrations of respective bases. By subjecting the Ointment formulations of *Allamanda cathartica* extract to Thermal Cycling studies under altered cycling temperature condition, no changes were found among all the ointment formulations. The results were shown in Table No. 8. Owing to all the results of physical parameters characterization, Ointment formulations of *Allamanda cathartica* extract containing Oleaginous bases (OF1, OF5) and Absorption bases (OF2, OF6) were not considered for further characterization. Ointment formulations of *Allamanda cathartica* extract containing Hydrophilic base (OF3, OF7) and PEG base (OF4, OF8) were subjected to *in vitro* drug diffusion studies and stability studies.

Stability study: There was no evidence of phase separation, development of disagreeable odour, change in colour, consistency and no change in parameters like pH, Viscosity, Spreadability of the all four products during stability study for three months.

***IN VITRO* Drug Release Studies:** Ointment formulations of *Allamanda cathartica* extract containing Hydrophilic base (OF3, OF7) and PEG base (OF4, OF8) were subjected to *in vitro* drug diffusion studies up to 8 hours abiding standard procedures through dialysis membrane (Sigma Inc. MO, USA; dry, unwashed, pre-cut and open ended; fiat width: 35 mm; inflated diameter, 21mm; Length: 30mm).

The results were shown in Table No. 9 and Figure No. 1. OF3 and OF4 formulations comprising low concentration (5%) of Hydrophilic and PEG bases have shown good dissolution characters by releasing 90.76% and 85.43% active principle.OF7 and OF8 formulations comprising high concentration (10%) of Hydrophilic and PEG bases have shown retarding effect by releasing only 65.34% and 52.51% of active principle, which may be due to their high consistency and viscosity of the formulations retarding the active principle without convenient diffusion.

Release Kinetics: By fitting the in vitro diffusion data into popular five regression and exponential models, all *Allamanda cathartica* Ointment formulations were found to be accepting zero order kinetics which was evident by higher R2 values of zero order plot compared to R2 values of first order plot that specifies independency of the concentration. *Allamanda cathartica* extract Ointment formulations were found to be following Diffusion mechanism, which were evident by higher R2 values of Higuchian plot compared to R2 values of Erosion plot. The plots were shown in Figures 2 to 6. In order to assess the exact release mechanism, dissolution data of AC Ointment formulations were fitted to Korsemeyer Pappas plot. All the exponent (n) values were found to be between 1.5 - 1, which indicates that the ointment formulations were exhibiting Anomalous (Non-Fickian) transport mechanism for the drug release at rate controlled fashion.

***IN VIVO* WOUND HEALING ACTIVITY ASSESSMENT:** The effect of optimized *Allamanda cathartica* ointment on Excision wound model was evaluated, the wound healing contracting ability in different formulations was significantly greater than that of control i.e. simple ointment treated group. Excision wound healing by contraction or wound closing and epithelization, the percentage of wound closure or closure rate were studied by recording the changes in wound area at fixed intervals of time. Gentamycin ointment showed more potent significant wound healing activity compared that of controlled group rats.

Table No. 1: Composition of Oleaginous Ointment Base

Components	Official quantity	Required quantity
White wax	5%	1 gm
White petrolatum	95%	19 gm

Table No. 2: Composition of Absorption Ointment Base

Components	Official quantity	Official quantity
Stearyl alcohol	3%	0.6 gm
White wax	9%	1.8 gm
White petrolatum	88%	12.6.6 gm

Table No. 3: Composition of Hydrophilic ointment Base

Components	Official quantity	Required quantity
Stearyl alcohol	25%	5 gm
White petrolatum	25%	5 gm
Sodium laryl sulphate	0.10%	0.02 gm
Propylene glycol	12%	2.5 ml
Water	Upto100 %	q.s

Table No. 4: Composition of PEG ointment Base

Components	Official quantity	Required quantity
PEG4000	40%	8 gm
PEG600	60%	12 gm

Table No. 5: Composition of 5%w/w Ointment

INGREDIENTS	OF1	OF2	OF3	OF4
Allamanda cathartica extract	0.50%	0.50%	0.50%	0.50%
Oleaginous base	9.5 gm	-	-	-
Absorption base	-	9.5 gm	-	-
Hydrophilic base	-	-	9.5 gm	-
PEG base	-	-	-	9.5 gm
Methyl paraben	0.10%	0.10%	0.10%	0.10%
Propyl paraben	0.05%	0.05%	0.05%	0.05%

Table No. 6: Composition of 10%w/w Ointment

INGREDIENTS	OF5	OF6	OF7	OF8
Allamanda cathartica extract	1%	1%	1%	1%
Oleaginous base	9 gm	-	-	-
Absorption base	-	9 gm	-	-
Hydrophilic base	-	-	9 gm	-
PEG base	-	-	-	9 gm
Methyl paraben	0.10%	0.10%	0.10%	0.10%
Propyl paraben	0.05%	0.05%	0.05%	0.05%

Table No. 7: Results of Phytochemical screening of crude extract

S. No.	Phytoconstituents	Petroleum ether extract
1	Carbohydrates	-
2	Glycosides	+
3	Alkaloids	+
4	Steroids	+
5	Triterpenoids	+
6	Fixed oils	-
7	Gums and Mucilages	-
8	Amino acids	+
9	Tannins	-
10	Flavanoids	-

Table No. 8: Evaluation of *Allamanda cathartica* Ointment Formulations

formulation	Ph 0.1%w/v	Spreadability (gm.cm / sec))	Viscosity (cps)	Extrudability	Thermal cycling	drug content (%)
F1	5.61	9.36	29250	Very Poor	No change	85.47
F2	5.82	8.72	26245	Poor	No change	82.12
F3	6.65	15.27	33870	Good	No change	93.18
F4	6.81	12.54	34450	Good	No change	92.14
F5	5.70	10.25	28290	Poor	No change	82.62
F6	5.41	9.76	27235	Poor	No change	89.64
F7	6.02	18.72	38500	Excellent	No change	96.50

Table No. 9: Cumulative *in vitro* Drug Diffusion Data

Time (Hrs)	Cumulative Percent Drug Release			
	OF3	OF4	OF7	OF8
1	10.12±0.11	15.22±0.32	8.07±0.54	9.54±0.25
2	35.28±0.56	30.89±0.67	12.96±0.20	12.06±1.05
3	55.82±0.68	40.64±1.11	20.64±0.10	15.33±1.22
4	65.33±0.36	68.34±0.35	30.74±1.15	19.68±0.40
6	75.81±1.09	78.22±0.12	40.86±0.85	36.80±0.18
8	90.76±0.54	85.43±0.76	65.34±1.05	52.51±1.01

Table No. 10: *In vivo* Wound Healing Activity Assessment

Group	Treatment	Post wounding day area in square mm						% Protection	Period of Epithelization
		1st Day	4th Day	7th Day	10th Day	13th Day	16th Day		
I	Control	499.5 ±4.34	447.0± 9.31	408.1±6 .06	260.9±1 .73	184.9±2. 46	138.34± 0.7	72.3	28.336
II	Gentamycin	493.2 ±9.24	413.5± 2.45**	305.7±1 .78***	116.4±5 .00***	46.51±1. 88***	0.0±0.0 ***	100	10.52
III	ACHB	507.3 ±1.79	425.7± 1.55*	344.8±2 .46***	212.8±6 .12***	94.97±2. 43***	36.17±0 .10***	92.87	16.22
IV	ACPEG	513.8 ±1.70	463.8± 1.44	345.4±2 .92***	239.1±7 .34	99.10±0. 382***	35.24±0 .08***	93.14	19.76

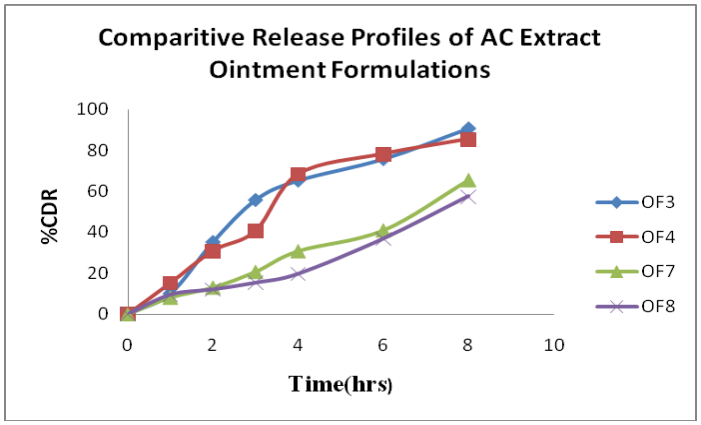

Figure No. 1: Comparative Release Profiles of OF3, OF4, OF7 and OF8 Formulations

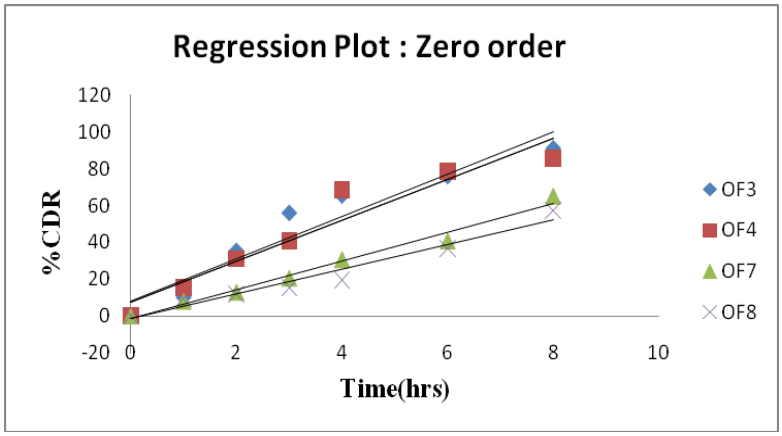

Figure No. 2 Zero Order Regression Plot for OF3, OF4, OF7 and OF8 Formulations

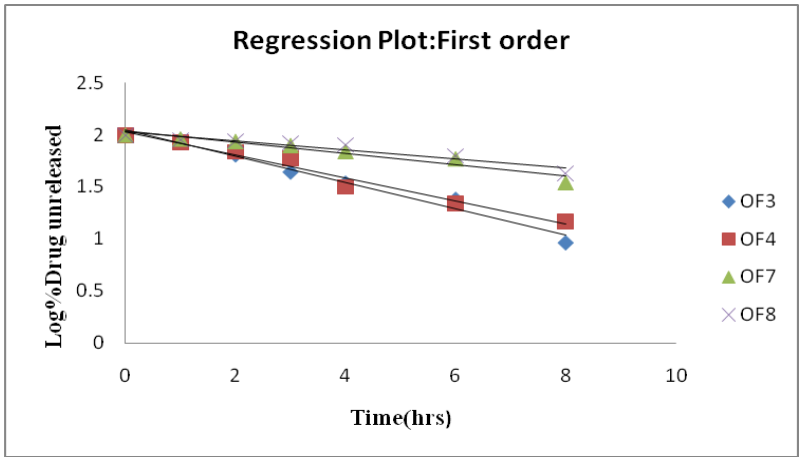

Figure No. 3 First Order Regression Plot for OF3, OF4, OF7 and OF8 Formulations

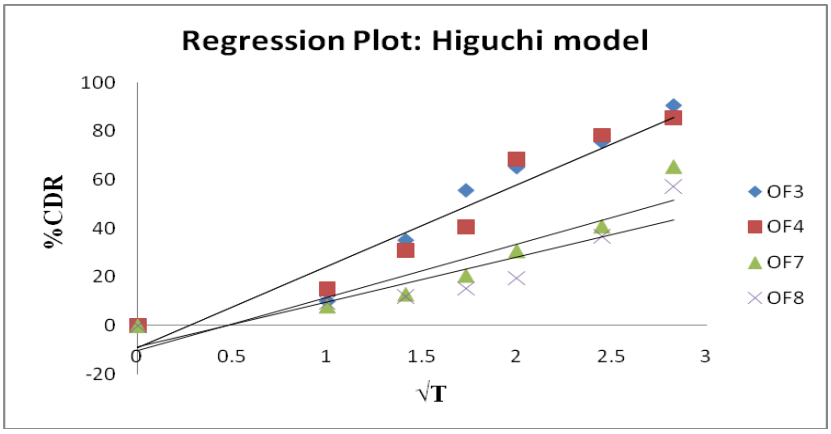

Figure No. 4: Higuchi Model Regression Plot for OF3, OF4, OF7 and OF8 Formulations

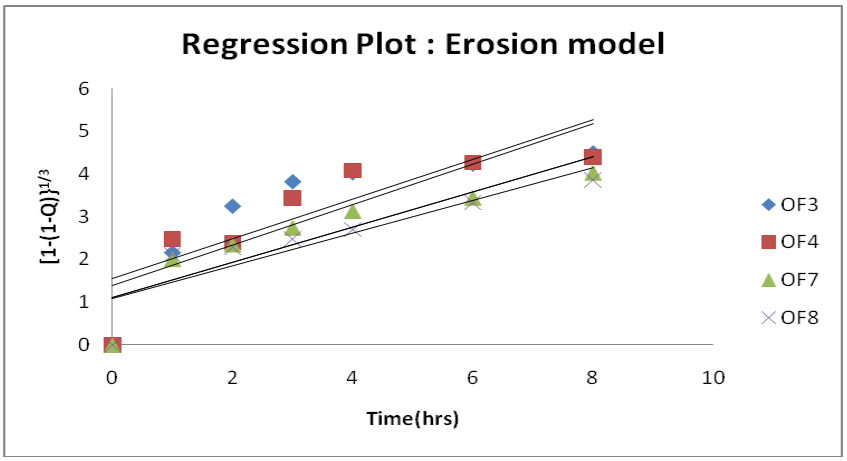

Figure No. 5: Erosion Model Regression Plot for OF3, OF4, OF7 and OF8 Formulations

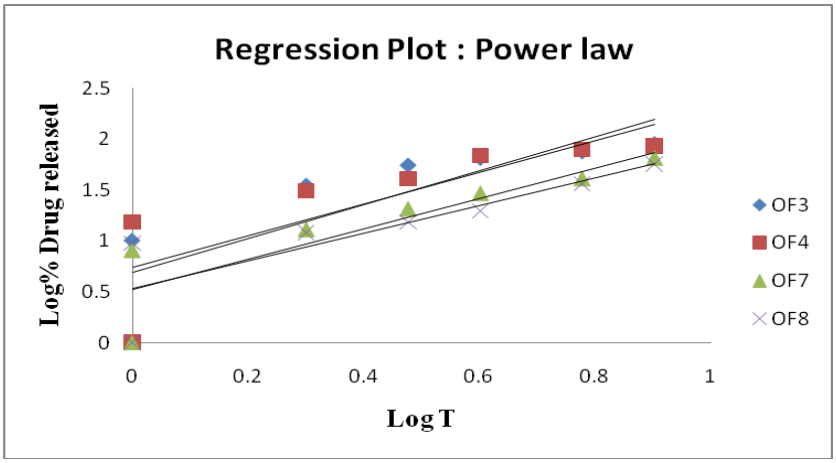

Figure No. 6: Pappas Model Regression Plot for OF3, OF4, OF7 and OF8 Formulations

AC HB showed better significant wound healing activity compared to AC PEG group rats. It was further found that all the four groups showed decreasing of wound area from date to day. However on 16th day, Group-I i.e. control group showed 72.3% protection, which may be due to self immunity of animals; whereas Group-

II i.e. standard group showed 100% protection. On the other hand Group-III i.e. ACHB treated group showed appreciable wound healing activity of 92.87% compared to standard group, whereas Group- IV i.e. ACPEG treated group exhibited 93.14% protection. ACPEG wound healing activity is closer to that of standard. Gentamycin indicating significant wound healing activity. All the results are showed in Table 10. Values were given as Mean ± SEM for six rats in each group values were statistically significant as compared with control.

CONCLUSION

Further Phytochemical studies are needed to isolate the active compounds responsible for wound healing activity. Further studies with purified constituents are needed to understand the complete mechanism of wound healing activity of AC leaves extract. Thus, this investigation confirms the use of both ACHB and ACPEG wound healing ointment preparations.

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