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Original article

Formulation and evaluation of anti-inflammatory herbal gel containing isolated solanesol

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Abstract

In the present study, we have made an attempt to formulate and evaluate an herbal gel containing solanesol. Solanesol was extracted from tobacco scrap and tested for its topical anti-inflammatory activity against carrageenan induced oedema. Carbopol-934 at 1% w/w concentration was used as gelling agent. The studies were conducted on male albino wistar rats (150-200 gm.). Change in oedema volume of the rat hind paw was measured. The anti-inflammatory effect produced after topical application of herbal gel formulation on carrageenan induced hind paw oedema exhibited a high degree of reproducibility. The initial physicochemical parameters of formulations, *i.e*., pH, viscosity, spreadability, extrudability and stability were also examined. The pH of all the formulations was near about 6.8, which lies in the normal pH range of the skin. Application of gel over rat skin did not produce any skin irritation, *i.e*., erythema and oedema for a month. The preparation was stable under normal storage conditions. The results of the study reveal the solanesol in the form of gel possess significant anti-inflammatory activity.

Key words: Solanesol, carragenan, carbopol, anti-inflammatory activity

1. Introduction

Plants have been one of the important sources of medicines ever since the dawn of human civilization. Chemically, medicinal plants may have secondary metabolites like alkaloids, glycosides, steroids or other groups of compounds which have marked pharmaceutical action as anticancer, antimalarial, anti-inflammatory, antidiabetic, antidysenteric, *etc*. In spite of tremendous developments in the field of allopathy during the 20th century, plants still remain as one of the major sources of drugs in modern as well as traditional system of medicine throughout the world (Biradar, 2015).

Solanesol is a polyisoprenoid alcohols or polyprenoils, was first isolated from flue-cured tabacco leaves by Rowland *et al.* (1956). It is found in many botanical species including tomato, potato, eggplants and pepper plants. In addition to this, the widely available crop 'tobacco' is the richest sources of solanesol. It is used for the systnesis of valuable chemicals like vitamin E, vitamin K and coenzyme Q10. Solanesol itself can be used as lipid antioxident and in the preparation of cardiac drugs; it is also stated that solanesol possesses antibacterial, anti-inflammatory and antiulcer properties.

Solanesol is a terpene compound and an important intermediate of nutrients such as vitamin K2 and co-enzyme Q10 (Shefali Srivastava *et al*., 2009). It is found in tobacco leaves and has curative effects against cardiac insufficiency (Sridevi *et al.,* 2015), muscular dystrophy, anaemia (Shefali Srivastava *et al*., 2009), cancer, diabetes, high blood pressure, asthma and liver injury (Yux Wang and Chen, 2008). Solanesol has been explored for various activities like antioxidant, antimicrobial, wound healing, antitumor, antihyperlipidemic, anti-inflammatory properties, *etc*. (Raveda and Lakshmareddy, 2009).

Inflammation is the protective mechanism of the local microcirculation to tissue injury which is caused by physical trauma, noxious stimuli by chemical agents, heat, antigen-antibody reaction and microbial effect (Raveda and Lakshmareddy, 2009). It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various methods for testing acute and subacute inflammation (Chaberlain *et al*., 1990). Carrageenan-induced paw oedema is a useful model to assess the contribution of mediators involved in vascular changes associated with acute inflammation (Stevenson and Narasimharao, 1963).

The popular steroidal and non-steroidal anti-inflammatory drugs are responsible for unwanted side effects (Sridevi *et al*., 2011). This necessitates the development of novel herbal drug formulations in the treatment of inflammation. Solanesol is one such phytochemical having potent anti-inflammatory properties (Chamberlain *et al*.,1963).

The present study was aimed to formulate and evaluate topical gel of solanesol and its *in vivo* anti-inflammatory activity.

2. Materials and Methods

Solanesol was extracted in-house from powdered tobacco leaves. Carbopol-934 was purchased from Sigma Aldrich. All other ingredients used were of analytical reagent grade.

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2.1 Extraction of solanesol

In the present research study, an important method has been introduced for the extraction of solanesol from the flue-cured tobacco leaves (Sato, 1975). In this method, the tobacco leaves were dried at 70°C for 3 h, grounded and passed through a 40-mesh sieve. The grounded leaf powder (1000 g) was extracted by employing n-hexane (5 lit) on a water bath at 50°C under refluxed for 3 h. and filtered. The residue was re-extracted successively with n-hexane. The hexane extracts were mixed and concentrated by rotary vacuum evaporator at 40°C. The pasty residue was saponified with 10% methanolic potassium hydroxide (50 ml) and then extracted with hexane, washed free of alkali, concentrated and again dried by rotary evaporation and it was eluted with 9:1 hexane: ethylacetate by column chromatography to get pure solanesol (Sheen *et al.*, 1978).

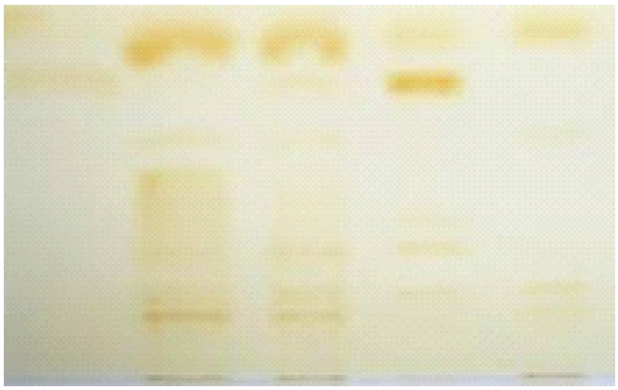


Figure 1: TLC chromatogram of solanesol

Column chromatography

Silica gel low-pressure column chromatography technique has been developed for the purification of the solanesol. In the present method, the crude solanesol dissolved in hexane at a ratio of 10:1 (v/w) of hexane-to-crude solanesol. It has been run on a silica gel column (30 cm×2.0 cm i.d.), which was pre-conditioned with n-hexane. The column was eluted with n-hexane : ethyl acetate (9:1, v/v). The eluent was collected in fraction of 5 ml and tentative identification has been carried out using TLC. The fractions containing solanesol was dried by rotary evaporation (Yux Wang and Chen, 2008) (Figures 1, 2).

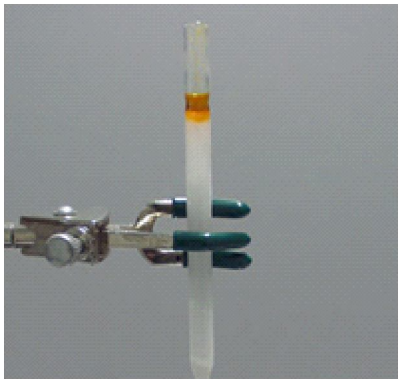


Figure 2: Silicagel column chromatography

2.2 Formulation of solanesol anti-inflammatory gel

Solanesol anti-inflammatory gel was prepared using a gelling agent Carbopol-934 (Table 1). Gelling agent concentration of 1% w/w was stirred with deionized water, using mechanical stirrer. The pH of the gel was adjusted to neutral, using triethanolamine with continuous stirring. Solanesol (1 % w/v) herbal extract was added to the gel and stirred for sufficient time to get homogeneous gel. Appropriate quantities of methyl paraben and propyl paraben were added with continuous stirring. Formulated gel was filled in collapsible tubes and stored in a cool and dry place until evaluation (Lipshutz, 2003).

Table 1: Formulation of solanesol gel

Ingredients	Quantities (% w/w)
Solanesol	1
Carbopol-934	1
Triethanolamne	QS
Methyl paraben	0.5
Propyl Paraben	0.2

2.3 Evaluation of solanesol anti-inflammatory gel

The extracted drug was characterized by IR, Mass and NMR studies (Figures 5, 6). The spectra obtained was analysed and data are shown in results. Formulated gels were evaluated for various physicochemical parameters such as colour, pH, homogeneity, viscosity, spreadability and drug content (Hideaka Fukuwa, 1970).

2.3.1 Measurement of pH

Five grams of gel formulation was dispersed separately in 45 ml of water, and the pH of the suspension was determined, using digital pH meter (Digital pH meter, Systronics, Noroda, Ahmedabad). Experiment was performed in triplicate and the average values were recorded (Hideaka Fukuwa, 1970) (Table 2).

2.3.2 Homogeneity

Gel formulations were tested for homogeneity visually after the preparation. Gels were tested for their appearance and presence of any aggregates (Hideaka Fukuwa, 1970).

2.3.3 Viscosity

The viscosity of gel was determined by using a Brookfield Viscometer DVII model with a T-Bar spindle in combination with a helipath stand. Fifty grams of gel was filled in a 100 ml beaker. T-bar spindle (T95) was used for the measurement of viscosity of all the gels. The helipath T-bar spindle was moved up and down and viscosity was measured at 10 rpm.

2.3.4 Spreadability

Spreadability was determined by wooden block and glass slide apparatus (Basha *et al.*, 2011). The apparatus consists of a wooden block, which was provided by a pulley at one end. By this method, spreadability was measured on the basis of slip and drag characteristics of formulations. An excess of the formulation (about 2 g) was placed on this ground slide and then formulation was sandwiched between this slide and another glass slide (movable) having the dimension of fixed ground slide and provided with the hook A weight of 1 kg was placed on the top of the two slides for 5 min to expel air and to provide a uniform film of the gel between

the slides. The top plate was then subjected to pull of 80 g with the help of string attached to the hook and the time (in seconds) required by the top slide to (movable) to separate completely from the fixed slides was noted. A shorter interval indicates better spreadability. Spreadability was calculated using the following formula (Basha *et al.*, 2011).

$$S = M \times L/T$$

where, S = Spreadability, M = Weight in the pan (tied to the upper slide), L = length of glass slide, T = Time (in sec) taken to separate the slide completely each other.

2.3.5 Drug content

Drug content was determined by dissolving accurately weighed 1 g of gel in phosphate buffer of pH 6 (Basha *et al.*, 2011). After suitable dilution, drug content was determined, using UV-visible spectrophotometer at 325 nm (Table 2).

2.3.6 FTIR studies

FTIR spectra are shown that there was no significant change in FTIR spectrum of the formulation compared to pure drug indicating absence of drug-excipient interaction (Figures 3, 4).

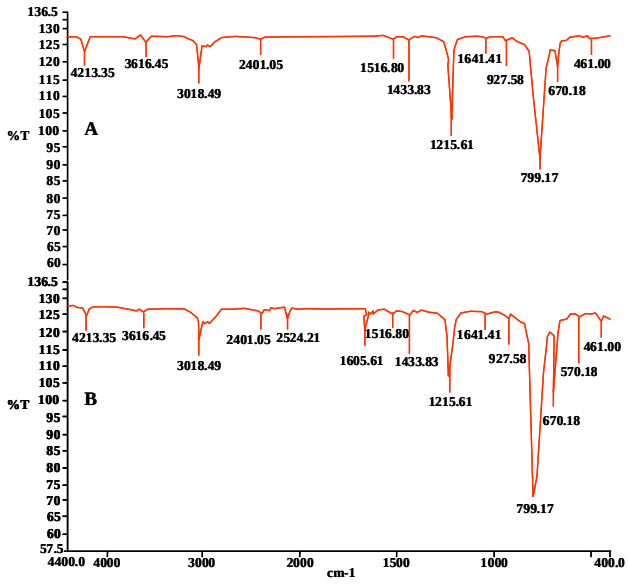


Figure 3: FTIR spectra of isolated solanesol (A) and gel formulation (B)

2.3.7 Stability studies

The formulated gel was subjected to stability testing as per ICH guidelines. The prepared gel was filled in collapsible tubes and stored at different temperatures and humidity conditions, *viz.*, 25±2ºC / 60±5% RH, 30±2 ºC / 65±5% RH, 40±2 ºC / 75±5% RH for a period of three months. Stored gel was periodically tested for its appearance, pH, spreadability and content uniformity (Mohapatra, 2008).

2.3.8 *In vivo* anti-inflammatory activity

The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) of Mallareddy College of Pharmacy, constituted under CPCSEA (1217/a/08/CPCSEA). Male albino rats weighing 150-200 g were used in the study (Shefali Srivastava *et al.*, 2009). Animals were allowed to free access to feed and water before the experiment. Animals were divided into three groups: control, standard and test with six animals in each group. Approximately 50 µl of a 1% suspension of carrageenan was prepared in saline 1h before each experiment and was injected into the plantar surface of the right hind paw of rat. To the test group, 0.2 g of gel containing solanesol was applied to the plantar surface of the right hind paw by gently rubbing with the index finger. Similarly, rats of the control groups received only the gel base. After one hour, topical preparation of solanesol and standard (50 µl of a 1% suspension of carrageenan in saline) was applied on plantar surface of the right hind paw of rat. Paw volume was measured after carrageenan application at 0h, 1h, 2h, 3h and 4h after the application, by using a plethysmometer, the paw volume was recorded at different time points. The percentage inhibition in paw volume was calculated by using the formula given below (Shefali Srivastava *et al.*, 2009).

$$\text{Percentage inhibition} = \left(\frac{A_0 - A_T}{A_0}\right) \times 100$$

2.4 Statistical analysis

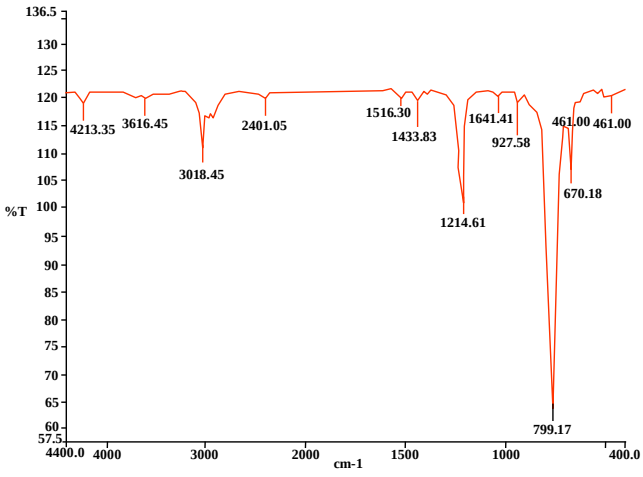
Analysis was performed using one-way analysis of variance (ANOVA) by "GraphPad Prism 5" software for determining the statistical significance between different groups.

3. Results and Discussion

3.1 Drug characterization

The extracted drug was characterized by IR, Mass and NMR studies. The spectral data are given below.

i. **IR (KBr)**-3616 cm^{-1} (OH), 3018 cm^{-1} (C-H), 1516 cm^{-1} (C=C), 670 cm^{-1} (CH Bending), 1433 cm^{-1}(C-C Strectching), 1215 cm^{-1} (C-O), 759 cm^{-1} (= C-H Bending).



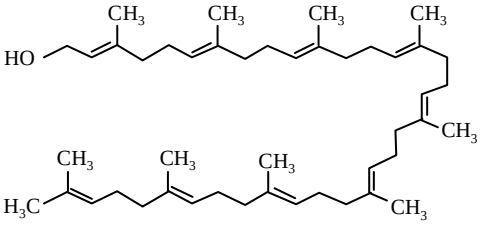
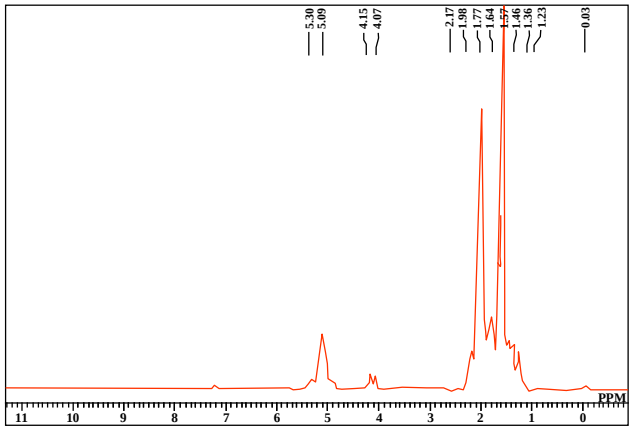


Figure 4: FTIR spectrum of solanesol

ii. 1H NMR (200MZ) (solvent: CDCl3) δ ppm: 1.36 (3H, 1CH3), 1.57 (21H, 7CH3), 1.64 (s, 3H) 1.77 (s, 3H), 1.98–2.17 (m, 32H), 4.07-4.15 (m, 2H, O–CH2), 5.09 (t, 8H) 5.30 (t, 1H).



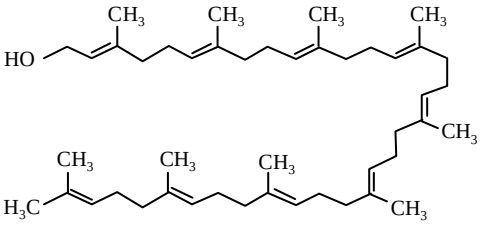
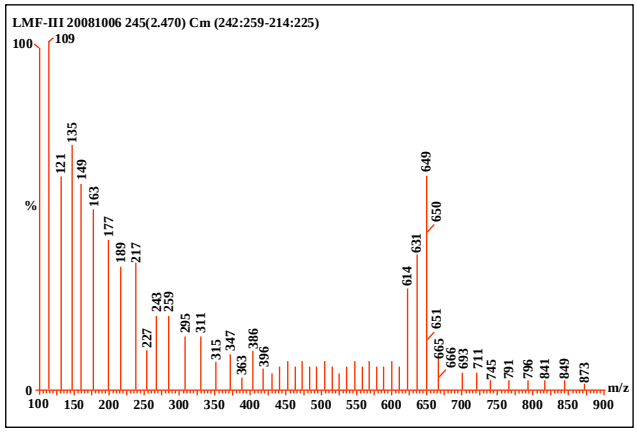


Figure 5: HNMR spectrum of solanesol

iii. MS (ESI) m/z: 649 (M+ + NH3).



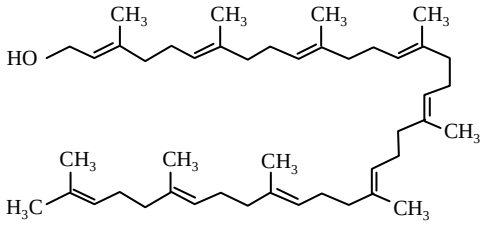


Figure 6: Mass spectrum of solanesol

All the prepared gel formulations were evaluated for various physicochemical parameters. Gel formulation was brownish and translucent in appearance. It had good homogeneity and spreadability. The pH of gel formulation was almost neutral (6.8±0.02). This was near to skin pH and acceptable for topical formulation. The viscosity of gel formulation was found to be optimum with good spreadability. Drug content of gel formulation was within the acceptable limits of IP. Results are shown in Table 2.

Table 2: Evaluation of solanesol gel

Evaluation Parameters	Results
Colour and appearance	Brownish and translucent
Homogeneity	Good
pH	6.8±0.02
Viscosity (cps)	9160±38
Spreadability (g.cm/sec)	22.40±2.16
Drug content (%)	96.3±1.32

3.2 Stability studies

Prepared formulation was subjected to stability studies as per ICH guidelines for three months. Stored gel was periodically tested for its appearance, pH, spreadability and content uniformity. There was no change in tested parameters at the end of 3 months, indicating the formulation containing solanesol was stable. Results of the stability studies are shown in Table 3.

Table 3: Stability studies

Parameters	0 M	1 M	2 M	3 M
Appearance	Brownish and translucent	Brownish and translucent	Brownish and translucent	Brownish and translucent
pH	6.8±0.12	6.9±0.22	6.9±0.66	6.9±0.42
Spreadability	21.42±2.46	22.62±2.26	21.22±1.88	21.51±5.24
Content uniformity	96.3±1.32	97.3±2.35	97.4±4.15	96.8±2.66

M = Month

3.3 *In vivo* anti-inflammatory activity of solanesol gel

Anti-inflammatory activity of selected gel formulation was investigated and results obtained are shown in Table 4.

In *in vivo* studies, there was no clinical signs of dermal toxicity were observed in any of the animals upon repeated applications. The changes were statistically insignificant. The statistical analysis of the evaluation of the anti-inflammatory activity of solanesol gel against the carrageenan induced paw oedema in albino rats were analyzed using ANOVA followed by Dunnett's *t*-test and expressed

as mean ± SEM (by using "GraphPad Prism 5" software). Differences between the mean of treated animals and control groups were considered significant at $p < 0.0001$. Carrageenan-induced hind paw oedema method exhibited a high degree of reproducibility. Solanesol gel showed percentage inhibition which is comparable to standard diclofenac sodium.

Table 4: *In vivo* anti-inflammatory activity of solanesol gel

Treatment	Dose (mg/kg)	Mean edema volume (ml) / Percentage inhibition				
		0h	1h	2h	3h	4h
Control	-	0.520 ± 0.0057	0.613 ± 0.0057 (8.5)	0.573 ± 0.0057 (14.4)	0.573 ± 0.0057 (14.4)	0.543 ± 0.0057 (18.9)
Diclofenac sodium	1 % w/w gel	0.223 ± 0.0057	0.236 ± .0.0057 (64.7).	0.200 ± 0.0100 (71.8).	0.176 ± 0.0057 (79.7)	0.163 ± 0.0057 (81.4)
Formulation	1 % w/w gel	0.310 ± 0.0057	0.316± .0.0057 (52.8)	0.276± 0.0057 (61.1)	0.200 ± 0.0100 (77.0)	0.176 ± 0.0057 (80.0)

The values are expressed as mean ± SEM, n=3 in each group (% inhibition). *p<0.0001 (Significant as compared with normal control).

4. Conclusion

Natural remedies are more acceptable to men as they are safer with few associated side effects than the synthetic ones. Demand for the herbal remedies in world market is continuously growing. In this study, we made an attempt to establish the anti-inflammatory property of solanesol in herbal gel formulation. Solanesol was isolated from tobacco scrap and it was confirmed by spectral analysis like UV, IR, Mass and HNMR. Solanesol topical gel was formulated, using Carbapol-934 as gelling agent. The formulated gel was evaluated for various *in vitro / in vivo* parameters in rats by carregenan induced rat paw oedema method. Results of the study revealed that the developed solanesol herbal gel formulation demonstrated comparable topical anti-inflammatory properties, supporting their traditional use for the treatment.

Conflict of interest

We declare that we have no conflict of interest.

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