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Formulation, characterization and *in vivo* evaluation of *Hedera helix* L., topical dosage forms

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Abstract: The purpose of this study was to prepare topical formulations of micro emulsion, gel and ointment containing the *Hedera helix* L. extracts against asthma and to evaluate the physicochemical characteristics. A validated HPLC method was used for the analysis of blood plasma. *In-vivo* studies of the drugs were compared in rabbit plasma with oral dosing. Stability studies were performed for 3 months. The results showed that formulations were stable. No Skin irritation observed on rabbits. The optimized micro emulsion and gel showed fast absorption. Maximal plasma concentration (c_{max}) and the maximal time to reach c_{max} (t_{max}) were 70.226µg/mL, 75.26µg/mL and 2 hours for the micro emulsion and gel, 90.11µg/mL and 1 hour for the oral drug syrup respectively. Pharmacokinetic parameters such as t_{max}, c_{max} and AUC of the selected formulations and oral dosing were significantly different ($P < 0.01$).

Keywords: *Hedera helix* L., *in vivo* study, rabbit plasma, topical formulations.

INTRODUCTION

Traditional herbal medicines are widely used in Asia but unfortunately these traditional ways suffer deficiency of stringent scientific evaluation. One example is the condition of asthma. Asthma is characterized by various air ways obstruction, inflammation, coughing, sneezing, itching and chest tightness. Asthma underlying causes including environmental promoters such as air pollutants, pollens, dust mites, allergens, genetic factors cigarette smoking, respiratory infectivity, seasonal cause and exercises (EPR, 2007). In allopathic system asthma was treated by corticosteroids, which have a range of adverse effects. One common side effect is the oral drug here to pass first pass effect. In Sothern Asia *Hedera helix* L. commonly used traditional herbal medicine among cultures.

Many species of *Hedera helix* L., genus are scattered in Balochistan, Pakistan. The biologically active components of the medicinal importance are triterpenoids, saponins, the hedercoside (B,C,F,G,H and F) and α-hederine (monodeside) have been isolated from *H. helix*. It has been revealed to be responsible for its β_2-adrenergic effects, which leads to the bronchodilatory, spasmolytic, mucolytic and expectorant action (Yulia *et al.,* 2012). The German commission verified therapeutic uses of the *Hedera helix* L., extracts for the acute catarrh of the respiratory tract with coughing and chronic bronchial inflammatory symptoms (Bluementhal, Busse, Goldberg, 1998).

A few activities are reported on the topical delivery of *Hedera helix* L., extracts such as using in the treatment of "Cellulites" Liprosclerosis and weight loss (Facino *et al.,* 1995). Lotions, cream and shampoos are used in skin (Wichtl, 2004). Also used as emollient and itch relieving, however, none of these established the *in vivo* study as topically applied formulations.

The specific objectives of the current research work were to formulate topical micro emulsion, gel and ointment and to evaluate for (*i*) different physico chemical characteristics, (*ii*) skin irritation study in rabbits, (*iii*) stability study as per ICH guide lines,(*iv*) *In-vivo* pharmacokinetic study compared to oral syrup.

MATERIALS AND METHODS

Chemicals

Hederacoside C was selected as the analytical standard for *Hedera helix* L. oleic acid and carbopol P934 were purchased from Merck, Germany. Wool fat, soft paraffin, cetostearyl alcohol, hard paraffin were purchased from Sigma Aldrich (USA). Acetonitrile, potassium dehydrogen phosphate, methanol, ethanol (95%), disodium hydrogen phosphate, and tri ethanolamine were purchased from Caledon. Acetic acid and Polysorbate80 were purchased from Fisher scientific. All other chemicals were used of high purity grade.

Plant collection and extraction from Hedera helix L.

The leaves of *Hedera helix* L. were collected from Quetta, Balochistan from June to September 2015. The plant was identified by Prof. Dr. Rasool Bakhsh Tareen, Professor of

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Extraction procedure

The extraction was done using Ibrar M (1998) method. 60 g dried plant material from *Hedera helix* L., was grind mechanically and weighed in the brown glass bottle and 900mL ethanol water 30 % was added. Kept for seven days in lab at room temperature with occasional shaking and then filtered. The filtrate was run in the rotary evaporator until a thick residue was achieved. This residue was washed in the separating funnel with ethyl ether to remove the fatty materials and chlorophyll. A thick viscous residue obtained was re dissolved in 200mL methanol with addition of ether in it. A saponin of white. –yellowish extract was formed. Ethyl ether was continued until no saponin was formed. The saponin was obtained by decantation and then airs dried at room temperature and yield the extract 8.4g.

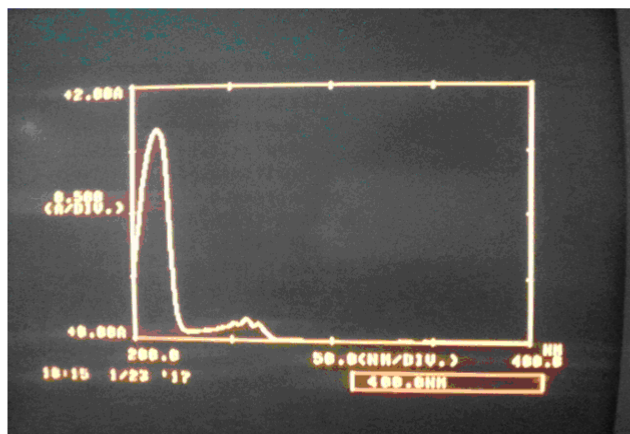

Fig. 1: UV-Visible Spectrum of Hederacoside C.

Analysis of hederacoside C

The HPLC method was performed as described by Suhaib and Zainab (2014), using Agilent Technologies System, 1100 Series, USA with LC- 10 AT VP pump, equipped with, DGU-AM 14 degasser, manual injector system, SPD-10 AVP UV-VIS detector and hypersil ODS C18 (150 X 4.6 mm) column. Agilent software was used for data processing and data collection. The chromatographic conditions used for analysis were as follows: The mobile phase consisted of (Acetonitrile: Water: Orthophosphoric acid) at ratios of 150:850:2 respectively. The flow rate was 1.0 mL/min, injected volume 20 µl and samples were detected at a wavelength of 210 nm. Each preparation contained 5 % (m/m) plant extract.

Preparation of microemulsion

The microemulsion was formulated using Chen *et al*, (2006) method. A mixture of Polysorbate 80 (Surfactant) and ethanol (co surfactant) was mixed in a ratio of 2:1. 4.6g aliquots of the surfactant mixture were added to the oil (oleic acid), 0.5g and were mixed with the magnetic stirrer properly. 0.5g plant extract was added in the above mentioned mixture and dissolved it completely finally, 4.4 g distilled water was added slowly under continuous stirring at 1200 rpm at room temperature. (Gul *et al*., 2017, 2017a and 2017b accepted).

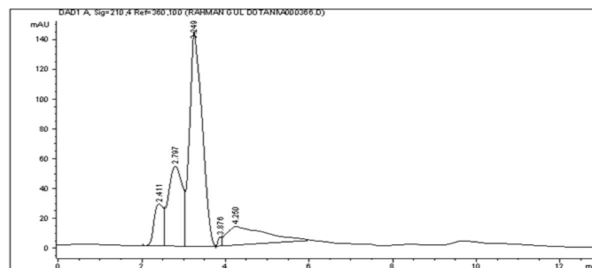

Fig. 2: Blank plasma sample of rabbit

Preparation of gel

Herbal gel was formulated using Proniuk *et al* method. 1g carbopol powder 934P was slowly dissolved in 50 ml distilled water with continuous stirring at 1200 rpm at room temperature. In next step, 0.5g plant extract was mixed in 10 ml ethanol and stirred until a homogeneous dispersion. The plant extract solution was added in the carbopol solution drop wise and stirred continuously, 2 ml olive oil was added to the final solution. Tri-ethanolamine (TEA) was added drop wise to initiate the gel formation and increase the pH. The final volume was made to 100 ml with sufficient quantity of distilled water and the gel was obtained (Gul *et al*., 2017, 2017a and 2017b accepted).

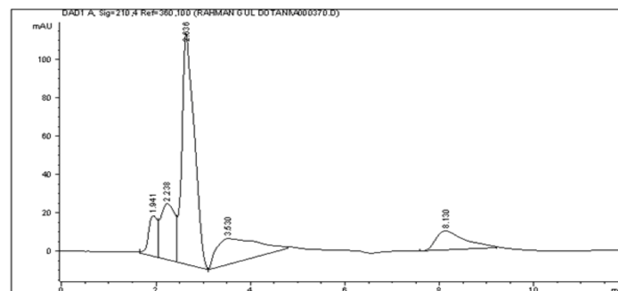

Fig. 3: Hederacoside C deducted from spiked rabbit plasma after administered orally.

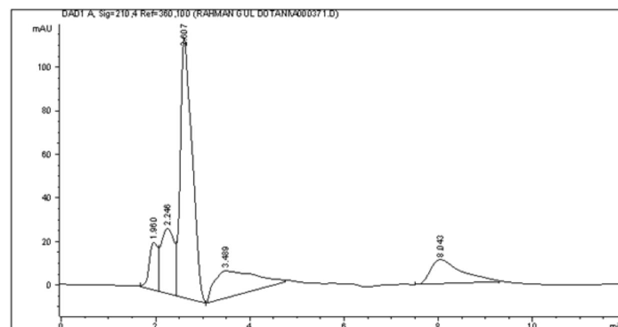

Fig. 4: Hederacoside C deducted from spiked rabbit plasma after administered topically.

Preparation of ointment
The formulation of simple B.P ointment was described by Marriot *et al*. First of all, the hard paraffin 4.75 g was melted at 60^0C. 4.75g. Wool fat was added Followed by 4.75g cetostearyl alcohol, stirred and cooled the prepared ointment at ambient temperature. Finally, 80.75g white soft paraffin and 5.0 g plant extract were added and the characterization was done (Gul *et al*., 2017, 2017a and 2017b accepted).

UV-Spectrum
10mg of hederacoside C was weighed exactly and transferred to a 100mL volumetric flask. The drug was sonicated and dissolved in methanol and made the volume up to 100mL with methanol. Further dilutions were made in ethyl alcohol and phosphate buffer (25:75) ratios. Resulting solutions were scanned as shown in fig. 1. In the range of 400 to 200 nm with the help of UV-Visible Spectrophotometer and λ_{max} was determined (UV-1600 Shimadzu).

Physico-chemical determination
The pH of all prepared formulations was checked by calibrated digital pH meter (Shivhere *et al.,* 2009). Viscosity of all formulations were determined at room temperature (25+/-1°C) using a cone and flat type viscometer (DV-III Ultra, Brookfield engineering laboratory, USA) (Kim *et al.,* 2003). Electrical conductivity of samples was tested using a conduct meter WTWcond197i (Weilhein, Germany) (Djordjevic *et al.,* 2005). Centrifugal tests were determined for all formulations immediately after preparations. For the centrifugation tests, 5g sample were carried out at 25°C for 10 min at 5000rpm as shown in table 1 (Ueda *et al.,* 2009).The droplet size analysis of the microemulsion from the plant extracts were determined by Zeta analyzer. Zetasizer Ver System; Malvern Instruments Ltd., Malvern, UK. Light scattering was monitored at 25^0C (Shafiq *et al*., 2007).

Spreadability
Spreadability of each formulation was assessed in term of diameter. In this process, 0.5 g formulation was kept over first glass slide. A second slide of 10g was kept over the first slide. The increased in diameter of circle in formulations and the diameter was measured the results shown in table 1 (Desai, 2004).

Skin Irritation Study
The formulations were applied to the rabbit skin in an area of almost $7.9cm^2$ and attached with gauze for duration of 1 h, and gauze was removed. At the end of the applied period, left over test substance was detached, without damaging the reaction of integrity of the skin. Observations were recorded after exclusion of the patch. The formulations were used to the skin once a day for a weak and reaction and sensitivity if any were observed and recorded as shown in table 2 (Parkash & Rao, 2010).

Stability studies
Stability studies were conducted at different storage temperature conditions for all formulations made from *Hedera helix* L. The Analysis were performed at sample kept at 0°C ± 1, 8°C ± 0.1°C in refrigerator, 25°C ± 0.1°C in incubator and 40°C±0.1°C in incubator. All formulations were tested for the change of appearance, pH, viscosity and conductivity (Shivhare *et al.*; 2009, Shinde *et al*.; 2005, Reddy *et al*., 2006, Gul *et al*., 2017).

In vivo permeability studies
Animal study was approved by Pharmacy Research Ethics Committee of the Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Pakistan. (Ref. No.29-2015/PREC).

Albino Rabbits of male sex weighing 2-3 kg were arranged from the animal house of Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur Pakistan. The animal were kept as per criteria of (FDA). The hair from dorsal region of the rabbits were shaved off with hair clipper and further it was freed from small hair by the application of hair removing cream (Anne French, Pakistan) one day before the application of test. The optimized microemulsion and gel 5g equivalent to (35 mg) were applied topically on the skin surface (7.9 cm^2), which was scrap with gauze. Blood samples of 1.0mL from vein of rabbits were withdrawn at 0, 0.5,1,2,3,4,6,8,12 and 24h in heprainized centrifuge test tubes after topical administration. Orally administered 5ml syrup equivalent to (35 mg) as the control of hederacoside C syrup for the pharmacokinetic studies, and the samples were withdrawn at 0,0.5,1,2,3,4,6,8,12 and 24h after dosing, in heprainized tubes. Immediately the collected blood samples were centrifuged at 5000 rpm then separated plasma layer was removed and stored at -70^0C until further analysis. The validated HPLC method was used for the drug analysis in each plasma sample.

Pharmacokinetics analysis
MS Excel 2007 and Kinetic software version 4.4 were used to analyze the pharmacokinetic parameters in *in-vivo* evaluation of plants extracts, topical formulations and marketed dosage forms and compared. Pharmacokinetic parameters evaluated include, optimum plasma concentration of drugs, (C_{max}), time to attain the maximum plasma concentration. (T_{max}), area under plasma concentration. time curve (AUC), and mean residence time, (MRT).

RESULTS

The Wave length maximum Absorption (λ_{max}) was found to be 210nm. as shown in fig. 1. The reported value is also 210.0 nm. This value was selected for the determination of drug contents in formulations and extract used in HPLC Technique.

HPLC (High Performance Liquid Chromatography)
The hederacoside C result quantified was 14.00% of the plant extract and the percentage of the active drug content of micro emulsion, gel and ointment formulation was 99.10%, 98.23% and 97.45 %.

The High Performance Liquid Chromatography technique was used for the deduction of hederacoside C in rabbit spike plasma. Samples were compared for their retention time and peaks area fig. 2. represent the chromatogram of blank plasma sample of rabbit. There is no variation in retention time of oral and topical formulations as shown in fig. 3 and fig. 4. The retention time remain in the range of 8.143, 8.043min in both dosage forms. The Pharmacokinetics concentration of both dosage forms were confirmed as shown in table.3.

Characterization results
The physicochemical characterization of the developed microemulsion, gel and ointments appear in table 1.

Stability study
The Stability of formulations kept at different storage conditions i.e. 0^oC, 8^oC, 25^oC and 40^oC was determined. For a period of 3 months at predetermined time intervals the three topical semi solid dosage forms were prepared from extracts of *Hedera helix* L. No previous data were available on the topical dosage made from the *Hedera helix* L., extract. No change in their physical characteristics including liquefaction, color, and phase separation and other parameters like pH, conductivity and viscosity were observed over a period of 90 days as shown in table 1.

Skin irritation test
In skin irritation study, no signs of edema and erythema were observed after 7 days after applications of microemulsion and gel in rabbits and are graded in table 2.

Pharmacokinetics studies
A validated HPLC method was used for the quantification of hederacoside C drugs in extracted plasma samples. Pharmacokinetics parameters were studied on the means plasma concentration against time profiles for topical micro emulsion, gel formulation and oral syrups after oral and topical administrations as shown in fig. 4. Results were tabulated and calculated Parameters were studied included maximum concentration drug in plasma (C_{max}) maximum time (T_{max}), area under curve. Time curve (AUC_{total}) mean residence time (MRT), half life ($T_{1/2}$) and total body clearance (CL) shown in table 4. Elaborated result *in vivo* study. The value of C_{max} from the optimized formulation microemulsion was 70.22 µg/mL and gel was 75.26 µg/mL of hederacoside C. Statistical data are expressed as mean ± standard deviation for five rabbits in each group p<0.05.

The time Taken of oral syrup C_{max} value at 90.11µg/mL of hederacoside C. The values of hederacoside c oral administration are almost similar to that of Ju Myung Kim *et al*., (2013). Who deducted the C_{max} at (30-300minutes) maximum plasma concentration. for optimized micro emulsion, gel, time mandatory to achieve utmost plasma concentration was 2 h for hederacoside C however, for oral syrup plasma concentrations was 1 h hederacoside C, this may be due to dermal accumulation of hederacoside C due to barrier of skin. An increased in the concentration the AUC for topical microemulsion, gel were (492.836, 985.35µg/ml/h), and for oral syrup, AUC was 329.38 µg/mL/h. lower than topical dosage form.

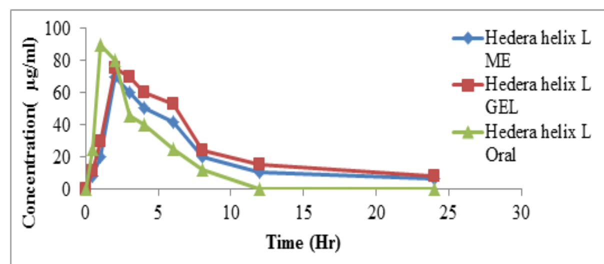

Fig. 5: Mean plasma concentration of Hederacoside C From oral and topical dosage forms micro emulsion, Gel formulations

Mean residence time of hederacoside C for micro emulsion, gel were 10.03 h, 3.85 h. However, mean residence time for oral syrup was 4.05 hour hederacoside C respectively, Total clearance of hederacoside C for micro emulsion, gel were 1.49, 1.07 1/h and oral syrup concentration was 2.21 1/h respectively, for topical micro emulsion and gel plasma level half-life of the hederacoside C 6.18, 10.71 h respectively, oral syrup 2.44h for hederacoside C. An evaluation of plasma drug concentration (µg/mL) of hederacoside C drugs against time in h for optimized micro emulsion, gel and oral syrup was prepared graphically in fig 5 for hederacoside C.

DISCUSSION

The physicochemical properties showed that all formulations were stable. It is visible from the data that the developed formulations had a low viscosity for microemulsion and gel and high viscosity of ointment. Our formulations were in good compliance with the skin pH. Normal human skin has a pH ranging from 4.5-6 (Panda, Ghosh, 2010). The mean droplet diameter was 110nm for hederacoside C. Which is in the range of normal microemulsion droplet size. Stability study was consistent with a previous study in which topical dosage forms prepared from an extract of Eupatorium odoratum L (Jennifer *et al*., 2003). In the topical dosage forms, product stability is one of the most important quality criteria. No indication of skin irritation was observed.

Table 1: Physical parameters values for *Hedera helix* L. formulations

Formulations	pH Mean±SD	Conductivity	Phase separation	Viscosity(cps) Mean±SD	Drug content (%) Mean±SD	Spreadability (g.cm/s) Mean±SD
Microemulsion	5.9±0.02	0.0	NO	16.8 ±0.001	99.10±0.03	5.6±0.02
Gel	5.7±0.04	0.2	NO	23.98±0.003	98.23±0.04	4.7±0.05
Ointment	5.8±0.02	0.0	NO	154.7±0.002	97.45±0.02	2.1±0.04

Mean±SD (n=3)

Table 2: Skin Irritation Study for 7 days results of formulations

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	A	A	A	A	A	A
Microemulsion	A	A	A	A	A	A	A
Gel	A	A	A	A	A	A	A
Ointment	A	A	A	A	A	A	A

A-No reactions, B-very Slight erythema, C-Moderate to severe erythema D-Moderate or severe edema

Table 3: Mean values of pharmacokinetics parameters of topical ME, Gel formulations and oral syrups

Pharmacokinetics Parameters	Transdermal Administration		Oral Administration
	Micro emulsion	Gel	
C_{max} (μg/ml)	70.226±0.219	75.26±0.152	90.11±0.224
T_{max}(h)	2	2	1
AUC_{Total}(μg/ml/h)	492.836±.316	985.35±1.121	329.38±0.453
MRT(h)	10.03±0.324	3.85±0.435	4.05±0.412
CL(1/h)	1.49±0.222	1.07±0.111	2.21±0.218
T(1/2)	6.18±0.214	10.71±0.332	2.44±0.321

C_{max}, maximum concentration; T_{max}, time to reach maximum concentration; AUC, area under the concentration-time curve; MRT, mean residence, CL, clearance; time;$T_{1/2,}$elimination half life.; ME, Microemulsion

Topical administration had a maximum plasma concentration and longer time. Higher mean residence time, and higher AUC as compared to the oral hederacoside C syrup Compared with oral administration, The method for the enhanced topical penetration of microemulsion, gel might be a attainable to numerous factors, first of all the concentration of formulation (5%) is resulted high concentration gradients, which might be the main method by which formulations penetrate the skin (Chen *et al*., 2006).

A number of studies have reported that improved topical delivery of drugs may be depend on a chaotic state, or depend on a unusual array of lipids given that intercellular lipids can reschedule into specific situation (Foldvari *et al.,* 2010).

Micro emulsion may perform as drug reservoirs whereby drug is released from inside to outside phase and then in to the skin or due to small globule size, droplet settling in close attachment with the skin might go through the skin surface (Peltola *et al*., 2003).

CONCLUSION

In this study, novel microemulsion, gel and ointment formulations for transdermal delivery of *Hedera helix* L., extracts were developed. The statistical data showed the formulations were physicochemically stable and nonirritant topically. An *in vivo* study in rabbits plasma significantly showed that the optimized formulations micro emulsion and gel enhanced the bioavailability compared the oral route of hederacoside C syrups. These formulations data together promote the suggestion that micro emulsion and gel formulations showed potential novel delivery systems to improve the release and skin permeability of hederacoside C.

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