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RESEARCH ARTICLE

Evaluation of transdermal administration of α-cyperone (4,11-selinadien-3-one) isolated from purple nutsedge (*Cyperus rotundus*) essential oils as a new drug delivery treatment method for lowering cholesterol

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Abstract

The compound α-cyperone (4, 11-selinadien-3-one) isolated from purple nutsedge (*Cyperus rotundus*) essential oil was investigated for cholesterol lowering effect. In this study, we removed the hair from the back area of the rats, this compound gave us a good result as an alternative drug for lowering serum cholesterol levels *via* the transdermal route of administration. This compound significantly decreased total serum cholesterol level in rats at $p \leq 0.01$.

Keywords: **Transdermal administration, cholesterol-lowering drug, *Cyperus rotundus*, liver**

Introduction

Cholesterol

Cholesterol plays a unique role among the many lipids in mammalian cells. This is based partly on its biophysical properties, which allow it to be inserted into or extracted from membranes relatively easily. Additionally, it plays an important role in organizing other lipids in a bilayer. Because of the importance of sterols, cells have evolved complex mechanisms to tightly regulate their abundance and distribution.
Since cholesterol homeostasis is critical at the whole body level, cells have various dedicated pathways for the uptake of cholesterol from low-density lipoproteins (LDL) and export to high-density lipoproteins (HDL). Most mammalian cells synthesize cholesterol endogenously, but it can also be delivered by lipoprotein carriers (Maxfield and van der Meer, 2010). Common cholesterol-lowering drugs include statins, fibrates, niacin, resins, and cholesterol absorption inhibitors. All of these drugs have side effects such as diarrhoea, constipation, stomach pain, cramps, bloating, nausea, vomiting, headaches, drowsiness, dizziness, muscle aches or weakness, flushing, and sleep problems (American Academy of Family Physicians, 2011).

Cyperus rotundus L. (Cyperaceae)

Cyperus rotundus (Cyperaceae) is a multipurpose plant, A number of pharmacological and biological activities including anti-candida, anti-inflammatory, anti-diabetic, anti-diarrhoeal, cytoprotective, anti-mutagenic, antimicrobial, antibacterial, antioxidant, cytotoxic and apoptotic, anti-pyretic and analgesic activities have been reported for this plant (Durate et al.,2005; Sundaram et al.,2008; Raut and Gaikwad, 2006; Uddin et al.,

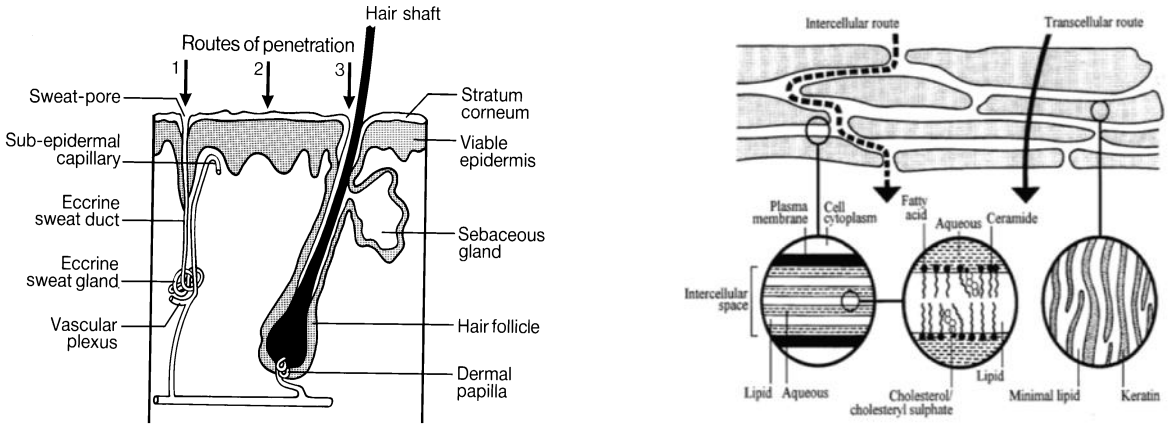
2006; Kilani et al. ,2005 ; Zhu et al., 1997; Kilani et al., 2007; Kilani et al., 2008; Dhillon et al., 1993; Pal and Dutta, 2006; Kilani et al., 2008; Sandeep et al., 2010; Sawanee et al., 2008 ;). It is a source of natural antioxidant (Nagulendran et al., 2007) and protective measure against mosquito bites by using the hexane extract of its tuber (Singh et al., 2009), and it has antinociceptive effect, but Poonam et al., 2004 had shown it has limited activity against different forms of infectious diarrhoea .

The skin and transdermal drug delivery (TDD)

The skin, also known as the integument or cutaneous layer, is the largest single organ of the body. It accounts for 15-20% of the total body weight and has a surface area of 1.5-2 m^2 in adults. The skin is composed of the epidermis, an epithelial layer of ectodermal origin consisting of keratinized squamous epithelium, and a dermal layer comprising mesodermal connective tissue. The highly vascular dermis nourishes and supports the epidermis and consists of a thick layer of dense, fibroelastic connective tissue, which contains many sensory receptors. Beneath the dermis lies the subcutaneous tissue or hypodermis, which is loose connective tissue that may contain pads of adipocytes (Mesher, 2010). TDD systems are used to topically administer medication in the form of patches that deliver drugs at a pre-determined and controlled rate for a systemic effect. Currently, about 74% of drugs are taken orally, but this route of administration is not always effective. TDD systems have emerged to improve drug delivery characteristics (Kumar et al., 2012).

The skin has been investigated as a route to deliver drugs topically, regionally, or systemically, but unfortunately dermis and TDD are often limited by poor drug permeability (Fang *et al*., 2003). Low permeability can be attributed mainly to the outermost layer of the skin (the stratum corneum), which serves as a rate-limiting lipophilic barrier against the uptake of chemical and biological toxins and loss of water (Shah, 1994; Hadgraft, 2004). The epidermal cell membranes in the stratum corneum are so tightly joined that there is hardly any intercellular space through which polar non-electrolyte molecules and ions can diffuse (Hsieh, 1994). The proteins and lipids of the stratum corneum form a complex interlocking structure, resembling bricks and lipid mortar (Shah, 1994). The major lipids found in the stratum corneum include cholesterol and fatty acids (Law *et al*., 1995). Ceramides, in particular ceramide 2 and ceramide 5, play an important role in the overall lipid matrix organization of the stratum corneum and in skin barrier function (Chen *et al*., 2000). Ceramides are tightly packed in lipid layers owing to the strong hydrogen bonding between opposing amide headgroups. This specifies a transverse organization in addition to the lateral orthorhombic chain organization of ceramide molecules. Different routes through which molecules can cross the stratum corneum include the transcellular, intercellular, and appendageal (i.e., through the eccrine/sweat glands or hair follicles) routes (Figure 1. by Barry, 2001).

Figure 1. Drug permission through the skin and Mechanism of skin permission (Barry, 2001)



Many studies have shown that the skin can absorb chemical compounds like coumarin. A study by Yourick and Bronaugh (1997) indicated that coumarin absorption is significant in the skin. Systemic coumarin absorption must be expected after dermal contact with coumarin-containing products. The use of coumarin in food was banned by the Food and Drug Administration because of reports that it induced hepatotoxicity in rodents. Yokoi et al. (2008) showed that rats topically treated with 0.42 mg GeO_2/g ointment had significantly higher germanium concentrations in the plasma, liver, and kidney than the corresponding concentrations in control rats. This finding indicates that the skin is permeable to inorganic germanium ions or germanate. Another study on the short-term dermal absorption and penetration of an organic chemical (dibromomethane) in aqueous solutions found that the amount of chemical in the skin and its fate during short exposures is important. The square-root-of-time approach predicted the total amount of chemical, which penetrated the skin and was absorbed, better than the steady-state approach (McDougal and Jurgens-Whitehead, 2001).

Chemical enhancers can be divided into two broad categories: those that change partitioning into the stratum corneum and those that influence diffusion across the stratum corneum (Thomas and Finnin, 2004). Examples of chemical penetration enhancers include sulfoxides (dimethylsulfoxide), alcohols (ethanol), polyols (propylene glycol), alkanes, fatty acids (oleic acid), esters, amines and amides (urea, dimethylacetamide, dimethylformamide and pyrrolidones), terpenes, cyclodextrins, surfactants (non-ionic, cationic, and anionic), and ozone (Walker and Smith, 1996; Foldvari, 2000).

TDD offers many advantages, such as reduced side effects, improved patient compliance, elimination of the first-pass metabolism, and sustained drug delivery (Park *et al.*, 2014; Singh and Morris, 2011). Terpenes are constituents of essential oils that are well-recognized penetration enhancers of drugs across the human skin, and have been receiving considerable interest in the pharmaceutical industry for this application (Cornwell and Barry, 1993). In general, they have low systemic toxicity and do not cause skin irritation, in addition to having good penetration-enhancing abilities (Cornwell et al., 1996). They are clinically acceptable and relatively safe skin penetration enhancers for both lipophilic and hydrophilic drugs (Gao and Singh, 1998). Terpenes are arguably the most advanced and established category of penetration enhancers and are classified as generally regarded as safe by the Food and Drug Administration (Aqil et al., 2007).

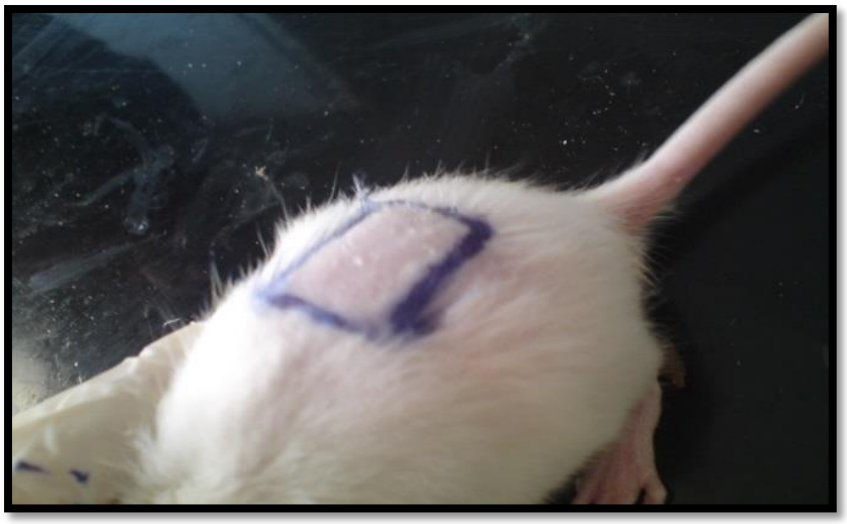
The aim of this study was to evaluate transdermal administration of α-cyperone (4,11-selinadien-3-one), which was isolated and identified from purple nutsedge (*Cyperus rotundus*) essential oil, as a new treatment method for lowering cholesterol.

Materials and Methods

Laboratory animals

White laboratory rats (*Rattus norvegicus*; n= 32) were housed under standard laboratory temperature (22-25 °C) and light conditions. The animals were divided into 2 groups (control group and treatment group). Each group consisted of 16 rats (8 male and 8 female). Hair on the dorsal area of each rat (approximately 2.5 cm^2) was removed by using sugar syrup (Figure 3). Animals in the control and treatment groups were treated topically with 0.1 mL of distilled water and 0.1 mL of 0.25 mg concentration α-cyperone (4,11-selinadien-3-one), respectively, twice daily for 30 days. At the end of the experiment, the rats were generally anesthetized by chloroform inhalation and then sacrificed. Blood samples were collected directly from the heart by using disposable 10-mL syringes. Then, blood samples were transferred into anticoagulant-free test tubes and allowed to clot at room temperature. Next, the samples were centrifuged at 3000 rpm for 15 min and the serum was collected and frozen at -20 °C until analysis. The animals were treated in accordance with the Ethical Guide for the Care and Use of Laboratory Animals (National Research Council, 2002).

Figure 2. Rat with removal area



Histological study

Tissue sections were prepared from the liver according to the method described by Luna (1968). Liver sections were isolated from the sacrificed animal, cut into small pieces, and prepared as follows: fixationin 10%, dehydration, clearing, impregnation, embedding, trimming and cutting, sectioning, mounting, and staining.

Statistical analysis

Data are expressed as the mean ± standard error of the mean (SEM) and were analyzed by two-way analysis of variance. Differences between means were considered significant at $P \leq 0.01$ using the Fisher's least significant difference (LSD) test.

Results and Discussion

The effect of α-cyperone (4,11-selinadien-3-one) is shown in Table 1. Compared to the control group, both male and female animals in the treatment groups showed significantly decreased serum cholesterol levels ($p \leq 0.01$). The decrease in serum cholesterol levels in males treated with α-cyperone (4,11-selinadien-3-one) was significantly higher than that in females, indicating that gender affected by the treatment.

Table 1. Effect of α-cyperone (4, 11-selinadien-3-one) on blood serum cholesterol levels in rats

Treatment	**Cholesterol, mmol\liter**		**Treatment Mean ± SEM mmol\liter**
	Females	**Males**	
Control	87.00 ± 5.49 a	88.40 ± 8.18 a	87.70 ± 4.76 A
α-Cyperone	81.30 ± 11.62 b	68.60 ± 5.48 c	75.00 ± 6.42 B
Mean of sex	84.15 ± 4.03 A	78.50 ± 15.00 B	
LSD	interaction	sex	
	0.75	2.21	0.41

* Capital letters indicate significantly different mean values ($P < 0.01$) for either treatment or sex. Small letters indicate interactions.

The results of our study showed that transdermal treatment with α-cyperone (4,11-selinadien-3-one) isolated from C. rotundus significantly decreased serum cholesterol levels. This compound dissolved in distilled water. The transdermal drug delivery (TDD) system provided an appealing alternative, minimizing the limitations associated with oral and parenteral drug administration (Alexander et al., 2012). The molecular mass of α-cyperone (218.34 Da) is within the range suggested by Barry, 2001, who showed that low molecular mass (<600 Da) was ideal for penetration of the stratum corneum, particularly when the molecule's diffusion coefficient was high and it had adequate solubility in oil and water . Cardiovascular disease accounts for a large proportion of total morbidity and mortality worldwide. Currently, the most common form of drug administration is the oral route. While this route has the notable advantage of easy administration, it also has significant drawbacks. These include poor bioavailability due to hepatic metabolism, and the tendency to produce rapid blood level spikes, necessitating high and/or frequent dosing, which can be both costly and inconvenient (Chien, 1992).

One long-standing approach for improving TDD involves the use of penetration enhancers. Essential oils were evaluated as penetration enhancers for 5-fluorouracil using excised human skin (Cornwell et al., 1993). *Eucalyptus* and *Chenopodium* species were very effective, causing an approximately 30-fold increase in the drug permeability coefficient. Ylang ylang was moderately effective (8-fold increase), while anise had less activity (3-fold increase) (Williams and Barry, 1989). Terpenes, which are found in essential oils, enhance skin penetration (Williams and Barry, 2012) and are popular choice as enhancers for TDD. For example, *l*-menthol has been used to facilitate the in vitro permeation of morphine hydrochloride through hairless rat skin (Morimoto et al., 2002), of imipramine hydrochloride across rat skin (Jain et al., 2002), and of hydrocortisone through hairless mouse skin (El-Kattan et al., 2000). Recently, niaouli oil was found to be the most the effective out of six essential oils in promoting estradiol penetration through hairless mouse skin (Monti et al, 2002). Notably, there is currently little regulatory control of the topical use of most terpenes, and many "aromatherapy" oils and formulations contain appreciable quantities of these enhancers. Their excessive use increases the potential for skin permeation of hazardous compounds from the same formulations.

α-Cyperone, one of the essential oil components present in *C. rotundus*, may enter the systemic circulation by penetrating the skin via hair follicles and the associated sebaceous glands, via sweat ducts, or across the

continuous stratum corneum between these appendages (Barry, 2001). This oil may penetrate the skin through intercellular or intracellular (transcellular) mechanisms (Barry and William, 1995). The mechanisms by which terpenes act on the lipid bilayer of the stratum corneum have been reviewed by (Jain et al., 2002).

Many basic aspects of cholesterol homeostasis are not well understood. Our results may be due to absorption of α-cyperone by the skin and its subsequent effect on proprotein convertase subtilisin/kexin type 9 (PCSK9). This protease has been identified from recent human genetic screens (Mousavi et al., 2009; Horton et al., 2009) and undergoes autocatalytic processing in the secretory pathway. The mature form is found in plasma, and it binds to the EGF AB domains of the LDL-receptor (LDLR), leading to its lysosomal degradation (Mousavi et al., 2009; Bottomley et al., 2009; Zhang et al., 2008 and Zhang et al., 2007). Introduction of PCSK9 into the circulation of mice through parabiosis reduced hepatic LDLR levels, which is consistent with PCSK9 interacting with LDLR on the cell surface (Lagace et al., 2006). Studies have shown that organic or inorganic compounds absorbed by the skin (Yokoi et al., 2008) can reach the liver through the systemic circulation and can affect liver metabolism. PCSK9 is expressed predominantly in the liver, small intestine, kidney, and brain (Seidah et al., 2003), and it is also present in human plasma (Lagace et al., 2006). Tavori et al. (2013) proposed that LDLR represented the main route of elimination of PCSK9 and that a reciprocal regulation between these two proteins controlled serum PCSK9 levels, hepatic LDLR expression, and serum LDL levels. Our data were consistent with this scenario, as the transdermal administration of α-cyperone may have decreased total blood serum cholesterol levels by inhibiting PCSK9-mediated LDLR degradation.

The binding of LDL to its receptor and its uptake by receptor-mediated endocytosis have been summarized in a recent review by (Goldstein and Brown, 2009). Alternatively, α-cyperone could affect PCSK9 mRNA expression. Cameron et al. (2008) investigated the effect of berberine, a natural plant extract, on PCSK9 expression in HepG2 cells. Berberine decreased PCSK9 mRNA and protein levels and increased LDLR mRNA expression in a time- and dose-dependent manner. The two possible mechanisms underlying this could be increased degradation of PCSK9 mRNA, or decreased transcription of the PCSK9 gene.

PCSK9 plays a critical role in cholesterol metabolism by enhancing degradation of the LDLR protein in the liver (Bedi et al., 2008); therefore, it is possible that α-cyperone could affect cholesterol metabolism. Another explanation is that α-cyperone may affect the cholesterol biosynthesis pathway. Zaragozic acid (ZA), a secondary metabolite produced by fungi, has good therapeutic potential because of its ability to decrease cholesterol biosynthesis. Bedi et al. (2008) showed that ZA administration could regulate hepatic expression of the PCSK9 gene in rats. Administration of ZA resulted in increased PCSK9 mRNA and protein levels in rat liver, and a concomitant increase in hepatic LDLR mRNA levels, LDLR protein turnover, and decreased serum cholesterol levels. Both the binding of PCSK9 to LDLR and its self-association are enhanced under the low pH conditions that are present in endosomes (Zhang et al., 2007; Fan et al., 2008). For many recycling receptors, occupancy by multivalent ligands can cause retention in the cell or delivery to late endosomes (Marsh et al., 1995). The mechanism of PCSK9-mediated regulation of LDLR is now a major target of therapeutic strategies for reducing circulating LDL.

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