Hydroalcoholic extract of *Sapium glandulatum* (Vell.) Pax displays potent anti-inflammatory activities through a glucocorticoid receptor-dependent pathway


**Abstract**

**Purpose:** The aim of the present study was to investigate the anti-inflammatory action of the hydroalcoholic extract of *Sapium glandulatum* (EHSG) leaves in mouse models of acute or chronic skin inflammation.

**Methods:** Topical effects of EHSG were evaluated in 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced edema in the ear. Systemic effects of the extract were studied in a TPA-induced ear edema model, as well as in a carrageenan-induced paw edema model. To gain insight into the mechanism by which EHSG blocked inflammation, we evaluated the role of glucocorticoid receptors (GR) using the TPA-induced ear edema model and also measured specific binding in a glucocorticoid assay. Possible adverse effects of EHSG were evaluated after multiple treatments with the extract in the skin atrophy model on the ear and with the alkaline comet assay.

**Results:** EHSG presented potent anti-inflammatory activity when applied topically in acute and chronic models, inhibiting edema formation and leukocyte migration as well as expression pro-inflammatory cytokines IL-1β, IL-6 and TNF-α in the tissue. Similar anti-inflammatory effects were found following oral treatment in both ear and paw edema models. Strikingly, the EHSG-induced blockade of leukocyte migration was reversed by mifepristone, a GR antagonist. Additionally, a specific binding assay revealed that EHSG interacts with GR. Multiple treatments with EHSG failed to induce adverse effects when evaluated in the skin atrophy model and bone marrow genotoxicity test.

**Conclusion:** Taken together, our data suggest that EHSG is a potential source of anti-inflammatory tool compounds for the treatment of pro-inflammatory-derived skin diseases, and its mechanism of action may be, at least in part, via the GR pathway.

**Keywords:** *Sapium glandulatum*, medicinal plants, skin inflammation, glucocorticoids

**Abbreviations:** EHSG, hydroalcoholic extract of *Sapium glandulatum* leaves; TPA, 12-O-tetradecanoylphorbol-13-acetate; MPO, myeloperoxidase; NAG, N-acetyl-β-D-glucosaminidase; IL, interleukin; TNF-α, tumor necrosis factor – α; PEG400, polyethylene glycol 400; ICAM-1, intercellular adhesion molecule 1; GR, glucocorticoid receptor.

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Introduction

Chronic inflammatory diseases of the skin, such as eczema and psoriasis, are very common and incurable, have considerable socio-economic impact and reduce the quality of life and self-esteem of patients (Wittmann et al., 2014). Treatment options may be limited because some existing drugs are ineffective in subgroups of patients who do not respond to treatment effectively or for whom the treatment is contraindicated due to side effects.
(Reich et al., 2014). For example, patients with atopic dermatitis require anti-inflammatory therapy based on topical glucocorticoids or calcineurin inhibitors. However, when these patients become refractory to topical treatment, systemic treatments become mandatory (Schakel et al., 2014). Patients with mild psoriatic plaques are successfully treated by topical glucocorticoids, emollients and vitamin D analogues. For moderate to severe psoriasis, systemic treatments are used, beginning with oral therapies, such as methotrexate, cyclosporine and sulfasalazine (Chang et al., 2011). Anti-TNF-α biological agents are successful in psoriasis with 75% efficacy in reducing symptoms, but not all patients respond adequately to treatment (Korn et al., 2009). Thus, there is still a need for new topical and systemic treatment options (Schakel et al., 2014). Besides, therapies that target a specific mediator in the skin, particularly when applied topically, can reduce systemic side effects compared to widely used immunosuppressants (Wittmann et al., 2014).

In this context, plants can be an important source of biologically active natural products and are considered a promising avenue for drug discovery due to their easy access and relatively low cost (Balanus and Kinghorn, 2005). Ethnobotanical studies of the Sapuía genus reveal that this genus is widely used in many countries for therapeutic purposes (Al Muqarrabun et al., 2014). Fu et al. (2013) demonstrated that topical application of ethyl acetate fractions from an ethanolic extract of S. sebhurum leaves inhibited edema formation in the TPA-induced ear edema model, this effect was mediated by antioxidant activity (Fu et al., 2013). The crude ethanolic extract of S. glandulosum roots showed low antioxidant activity and an extract of the leaves showed good antioxidant activity (da Silva et al., 2011). In another study, extract of S. japonicum leaves and branches were shown to inhibit lipopolysaccharide-induced nitric oxide production (Kim et al., 2010).

However, many species of the Sapuía genus have not been explored yet; actually, only 6 of 23 species have phytochemical studies, and a great chance remains to discover new bioactive chemical compounds with promising medicinal properties in these species (Al Muqarrabun et al., 2014), such as agents for the treatment of skin diseases. Several flavonoids and terpenoids, which have great potential for therapeutic use, including anti-inflammatory, immunomodulatory and antioxidant activities (Cragg and Newman, 2013), have been isolated from Sapuía plants (Al Muqarrabun et al., 2014).

The native Brazilian species S. glandulosum (Vell.) Pax leaves were collected in January of 2011 and 2013, in the morning, at the Nature Reserve in Rio Cachoeira, which is protected by Sociedade de Pesquisa em Vida Selvagem e Educação Ambiental (SPVS), and is located in the municipality of Antonina, Paraná, Brazil. The plant was identified by Dr. Katia Christina Zuffellato-Ribas and a copy can be found cataloged under the number CFC 9204 in the Herbarium of Forest Engineering, Federal University of Paraná.

**Extract collection**

A S. glandulosum hydroalcoholic extract 90% (EHSG) was prepared from the leaves of the plant. They were dried in a drying oven at 37°C, crushed (20 g) and subjected to extraction with 90% GL ethanol, at a 1:20 ratio (w/v) under agitation (330 rpm) at room temperature for 4 h. The product was filtered, concentrated in a rotary evaporator, lyophilized and stored in amber vials at 4°C until use.

**HPLC analysis of EHSG**

A Shimadzu LC-10AD LC system (Shimadzu, Tokyo, Japan) consisting of a binary pump and a Shimadzu SPD-M10A photo diode array detector were used to analyze the EHSG (2 mg/ml in methanol). The injections (20 µl) were made using an autosampler and carried out on a Phenomenex kinetex column (690 mm², 2.6 µm; Torrance, USA), and the column temperature was set at 45°C. The mobile phases were (A) 0.125% formic acid and (B) methanol. The gradient was as follows: 90:10 (A:B) (0–5 min); 85:15 (5–10 min); 75:25 (10–15 min); 72:28 (15–20 min); 65:35 (20–25 min); 60:40 (25–30 min); 55:45 (30–35 min); 50:50 (35–40 min); 35:65 (40–45 min); 25:75 (45–50 min); 15:85 (50–55 min); 0:100 (55–60 min); 90:10 (60–65 min). The flow rate was 0.7 ml/min, with detection at 300 nm.

**Animals**

All procedures were carried out on adult male Swiss mice weighing 25–35 g, randomly allocated in different groups. Food and water were supplied ad libitum and animals were kept on a 12 h light/dark cycle in a temperature controlled room (22 ± 2°C). All animal procedures were performed after protocol approval by the Institutional Ethics Committee of the Federal University of Paraná and were carried out in accordance with current guidelines for the care of laboratory animals, under protocol number 390.

**Acute ear inflammation by TPA**

Acute ear skin inflammation was induced with 12-O-tetradecanoylphorbol-13-acetate (TPA) (2.5 mg/ear) on the right ear of the mice. EHSG (0.03–1 mg/ear) and dexamethasone (0.1 mg/ear) were applied immediately following TPA. TPA and dexamethasone were diluted in 20 µl acetone, EHSG was diluted 20 µl of vehicle (18 µl acetone, 2 µl water). Ear thickness was measured before and 6 h after induction with a digital micrometer (Gabor, 2000). After 6 (peak of edema) or 24 h (peak of cellular infiltration), animals were euthanized and ear biopsies (6 mm) were collected and used to quantify cytokine levels (6 h after induction of inflammation) and assess the myeloperoxidase (MPO) activity or for histological analysis (24 h after induction of inflammation), respectively.

To evaluate the possible role of glucocorticoid receptors in EHSG activity, animals were pretreated with Mifepristone (50 mg/kg, s.c.) in polyethylene glycol 400 (PEG400), 15 min before TPA, and then the procedures were followed as described above.

In another set of experiments, animals were treated with EHSG (1, 10 or 100 mg/kg, p.o.) or dexamethasone (3 mg/kg, p.o.) 1 h before the TPA. EHSG and dexamethasone were dissolved in 0.9% saline. After 24 h, animals were euthanized and ear biopsies were submitted to an evaluation of MPO activity.

**Chronic ear inflammation by TPA**

Chronic ear skin inflammation was induced with TPA (2.0 mg/ear) on the right ear of the mice on alternate days for nine days. EHSG (1 mg/ear) and dexamethasone (0.1 mg/ear) topical treatments were applied twice per day (12 h/12 h), starting on the fifth day of the experiment. Ear thickness was measured daily and on the ninth day of the experiment the animals were euthanized and ear biopsies were collected, weighed and further evaluated (Gabor, 2000).
Acute paw inflammation by carrageenan

Acute paw inflammation was induced with carrageenan (300 μg/paw in 50 μl saline, i.pl.) subplantar administration on the right hind paw of the mice. EHSG (1, 10 and 100 mg/kg, p.o.) or dexamethasone (3 mg/kg, p.o.) was administered 1 h before carrageenan. Paw thickness was measured before and every hour after a 6 h induction. After 24 h, animals were euthanized and paw biopsies (6 mm) were submitted to an evaluation of MPO activity (Castardo et al., 2008).

MPO and N-acetyl-β-D-glucosaminidase (NAG) enzymatic activity assay

MPO and NAG enzymatic activity assays were performed as described by Mendes et al. (2012). To evaluate the EHSG effect on MPO enzyme activity in vitro, a homogenate with a high concentration of enzyme was used from mice ear samples subjected to multiple applications of TPA. A 20 μl aliquot of EHSG (0.01–300 μg/ml) was incubated with 30 μl of homogenate for 15 min and followed as described in the original method.

Histological assessment

Collected ear samples were fixed in ALFAC solution (80% ethanol, 40% formalin and glacial acetic acid). The ears were then dehydrated, embedded in paraffin, and sectioned into 5 μm slices. Slices were hydrated in xylene and a descending sequence of ethanol, then stained with hematoxylin and eosin. To evaluate the number of leukocytes, slices were photographed at a magnification of 400× and the photographs were analyzed with the ImageJ® software version 1.48 (National Institute of Health, USA).

Cytokine quantification

To quantify the levels of cytokines IL-1β, IL-6 and TNF-α, ear samples were homogenized in 2 ml of specific buffer (PBS, 0.05% Tween 20, 0.1 mM PMSF and 0.5% BSA) for 45 s at 0 °C. Homogenates were centrifuged at 3000 × g, 4 °C for 10 min. Detection of cytokine levels was performed with an enzyme-linked immunosorbent assay kit assay (ELISA) (Ready-Set-Go®, 88–7013, 88–7064 and 88–7324, eBioscience, Inc., San Diego, USA) according to the manufacturer’s instructions.

Evaluation of the effect of multiple topical EHSG treatments on skin atrophy and lymphoid organ weights

Animals were topically treated with dexamethasone (0.1 mg/ear) or EHSG (1 mg/ear) every 12 h for seven days, and ear thickness was evaluated daily. At the end of the experiment the animals were weighed, made blood glucose test and euthanized, and then the thymus, spleen, adrenals and auricular lymph nodes were collected and weighed. The glucocorticoid receptor binding assay was performed as described by Ferreira et al. (2005).

Alkaline comet assay

Bone marrow from the right hind paw femur extracted of animals submitted to multiple topical treatments with EHSG or dexamethasone was used in these experiments. For the control group, the animals received a single dose of cyclophosphamide (50 mg/kg, i.p.) 24 h before euthanasia. Bone marrow was washed with 4 °C PBS, pH 7.4, using a 1 ml syringe and separated. The resulting suspension was centrifuged at 1000 rpm for 10 min, the supernatant was discarded and 100 μl of 4 °C PBS, pH 7.4, was added and mixed. Sample (45 μl) was mixed with 120 μl of 0.5% low melting point agarose at 37 °C and spread on slides coated with 1.5% normal melting point agarose. Samples were protected from light at 4 °C for 20 min. After solidification, slides were immersed in cold, freshly prepared lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH 10 with 10% DMSO and 1% Triton X-100) for 2 h at 4 °C. After lysis, slides were placed in a horizontal electrophoresis unit containing fresh cold alkaline electrophoresis buffer (1 mM NaOH, 300 mM EDTA, pH > 13) for 20 min at 4 °C for DNA unwinding and conversion of alkali labile sites to a single chain. Alkaline electrophoresis was performed using the same alkaline electrophoresis buffer for 25 min at 30 V (0.8 V/cm) and 300 mA and 4 °C. Slides were washed 3 times for 5 min with neutralization buffer (0.4 M Tris, pH 7.5). After drying at room temperature, the

![Fig. 1](image-url) Representative HPLC chromatogram of (A) quercitrin (100 μg/ml); (B) EHSG (2 mg/ml); detected at 300 nm with insert of UV absorption profile of the major peak (peak 1, quercitrin).
slides were fixed in 100% ethanol for 10 min, dried and stored overnight. Finally, slides were stained with 45 ml of ethidium bromide (20 μg/ml). Slides were evaluated with 400x magnification using a fluorescence microscope (Olympus DP72, software CellF) with an excitation filter of 515–560 nm and a 590 nm barrier filter. Only individual cells were measured. Two slides were analyzed per sample and 150 cells per animal were samples according to the methodology of Hartmann and Speit (1997).

**Statistical analysis**

Results are presented as mean ± S.E.M. Statistical significance between groups was assessed by means of a one-way analysis of
variance (ANOVA) followed by a post-hoc Newman–Keuls test. The accepted level of significance for the tests was $P < 0.05$. All tests were carried out using the GraphPad Prism version 6.0c statistical software (La Jolla, California, USA).

**Results**

**HPLC analysis from EHSG**

The chromatographic profile of the extract (Fig. 1B) showed a major peak at the same retention time of quercitrin (Fig. 1A), with a UV absorption profile (insert of the figure) typical of flavonoids. The other minor peaks between 27–30 min, which were unidentified, also showed similar UV absorption profiles (data not show).

**Anti-inflammatory effects of topical EHSG on acute models of inflammation**

Topical application of EHSG was able to inhibit edema formation in a dose-dependent manner (Fig. 2A), and inhibited the enzymatic activity of MPO *in vivo* (Fig. 2B) and *in vitro* (Fig. 2C). The magnitude of inhibition was comparable with the reference drug dexamethasone, when compared with the control group. As determined through histology analysis, EHSG and dexamethasone reduced the number of migrating cells. Furthermore, application of the extract inhibited IL-1β, IL-6 and TNF-α cytokines levels (Fig. 3).

**Anti-inflammatory effects of oral EHSG on acute models of inflammation**

Orally administered EHSG also was able to inhibit significantly ear edema (Fig. 4A) and MPO activity (Fig. 4B). Additionally, dexamethasone inhibited edema and MPO activity as compared with the control group.

Four hours after carrageenan injection, paw edema reached the peak (control group) and EHSG prevented the edema at all doses tested (Fig. 4C). In the same model, EHSG significantly reduced MPO activity, but was less potent than dexamethasone (Fig. 4D).

**Anti-inflammatory effects of topical EHSG in a chronic model of inflammation**

Repeated application of EHSG started to reduce edema on the seventh day of the experiment, reaching the maximum decrease at the end of experiment, as compared with the vehicle group. The reference drug dexamethasone began to reduce edema on the fifth day through the ninth day (Fig. 5A). An increase in MPO and NAG activity in the vehicle group was caused by recurrent applications of TPA, and EHSG usage decreased MPO and NAG activity, as did dexamethasone (Fig. 5C and D). Treatment with EHSG also reduced the tissue levels of IL-1β and TNF-α in comparison with the vehicle group (Fig. 5E and F).

**Evaluation of glucocorticoid-like effects of EHSG**

Pre-treatment with the corticoid antagonist mifepristone caused no change in ear edema induced by TPA. Still, topical EHSG and dexamethasone inhibited the edema when compared with the TPA/PEG400 group. Pre-treatment with mifepristone did not modify the inhibitory response of EHSG on edema induced by TPA. Actually, mifepristone treatment was able to reverse the inhibitory activity of dexamethasone on ear edema, when compared with the dexamethasone/PEG400 group (Fig. 6A).

The increase in MPO activity was reduced by either EHSG or dexamethasone in comparison to the TPA/PEG400 group. Actually, previous treatment with mifepristone was able to reverse the enzymatic activity inhibition caused by EHSG and dexamethasone (Fig. 6B). Analysis *in vitro* showed that EHSG was able to reverse the binding of [1H]-dexamethasone only in the highest
concentrations tested, while non-radiolabeled dexamethasone reversed the specific binding of [3H]-dexamethasone at the tested concentration (Fig. 6C).

Topical application of dexamethasone for seven days caused alterations in the animals' appearance and behavior. They became lethargic and had more bristling hairs, which was not observed with EHSG administration (parameters evaluated subjectively by visual analysis). The treatments did not affect body weight (Fig. 7A) and blood glucose levels (Fig. 7B) of animals. Dexamethasone reduced ear thickness, whereas EHSG did affect this parameter (Fig. 7C). Both EHSG and dexamethasone treatments were associated with a decrease in weight of the thymus, spleen, adrenals and lymph nodes (Fig. 8A-E).

In the genotoxicity test, the positive control group showed an increase in DNA damage when compared with the group that received only vehicle, but no chromosomal damage was detected in the EHSG and dexamethasone groups (Fig. 8D).

**Discussion**

EHSG showed good anti-edema activity; it inhibited TPA-induced ear edema and carrageenan-induced paw edema in mice, both systemically and by local application. TPA promotes direct activation of protein kinase C (PKC), and in sequence activates mitogen-activated protein kinases (MAPK), phospholipase A2 (PLA2), induction of cyclooxygenase-2 (COX-2), expression and translocation/activation of lipoxygenase (LOX), thereby activating the synthesis and release of various proinflammatory mediators responsible for edema formation and leukocyte migration into the dermis (Murakawa et al., 2006). Carrageenan injection releases many mediators that operate in sequence to produce inflammation. Histamine, serotonin and bradykinin are the first mediators of inflammation in the early stage. The second phase involves high production of prostaglandin, induced by COX-2 and high neutrophil infiltration, associated with elevated levels of cytokines TNF-α, IL-1 and IL-6 (Necas and Bartosikova, 2013). It has been reported that plants of the Sapium genus contain various kinds of chemical compounds, mainly including the flavonoid and terpenoid classes (Al Muqarrabun et al., 2014). EHSG is no different, having a quercitrin as a major component of its composition. Several flavonoids, such as quercetins, are reported to show anti-inflammatory activity in vitro and in vivo. Although not fully understood, several mechanisms of action are proposed to explain the anti-inflammatory action of flavonoids in vivo. The most important mechanism could be the inhibition of the eicosanoid pathway, which includes phospholipase A2, cyclooxygenase and lipoxygenase, thus reducing the concentration of prostanoids and leukotrienes (Kim et al., 2004).
as well as other mediators of inflammation such as cytokines, chemokines and adhesion molecules (Tunon et al., 2009).

EHSG also reduced leukocyte migration to inflamed tissue and MPO activity, an indirect evaluation of neutrophil resettlement. In fact, inhibition of MPO activity was detected directly in an in vitro assay; however, the in vitro inhibition of the MPO enzyme activity was less pronounced than the reduction of the activity observed in vivo, suggesting that direct reduction of enzyme activity may not be the primary mechanism involved.

Indeed, cell infiltration to the inflamed site is indirectly promoted by cytokines. Keratinocytes respond to a stimulus like cytokine IL-1α and produce more IL-1α, and IL-1β, TNF-α and IL-6, promoting and amplifying the initial signal of inflammation, which, once reaching the dermis, stimulates fibroblasts to produce more of these cytokines and growth factors that in turn activate endothelial cells to express several adhesion molecules (E-selectin, P-selectin, ICAM-1, VCAM-1) (Spellberg, 2000). Furthermore, it has been demonstrated that topical TPA increases TNF-α levels locally.
and etanercept (TNF-α antagonist), inhibits edema but not cellular infiltration (Murakawa et al., 2006). Moreover, IL-6 is responsible for increasing the expression of adhesion molecules such as VCAM-1 and ICAM-1 in inflamed sites and endothelial cells and induces the production of chemokines, increasing neutrophil transmigration to the inflamed site (Mihara et al., 2012). Therefore, the reduction of key cytokines (IL-1β, TNF-α and IL-6) in TPA-induced inflammation can be one of the major routes by which EHSG promotes its anti-inflammatory effect. It is possible that the reduction in edema formation is originated by its actions to reduce TNF-α levels, and together with the diminution of leukocyte migration caused by IL-6 inhibition, promotes the anti-inflammatory action.

Another highlight of these results is that EHSG was less effective orally than topically. It is possible that the available concentrations of compounds with anti-inflammatory activity after oral dosing are smaller, since they would be subjected to metabolism and excretion. These data demonstrate that the choice of administration route is of great importance to the success of treatment, and in the case of EHSG, the topical route was more effective.

The anti-inflammatory action of EHSG importantly was confirmed in the animal model of repeated application of TPA on the ear. The extract showed influence on all parameters observed in this method: edema, MPO and NAG activity, as well as in levels of IL-1β and TNF-α, demonstrating its effectiveness on an established inflammatory process. Due to the fact that the EHSG reduces all parameters analyzed in the acute and chronic models just as dexamethasone did, despite being less effective in the chronic model, it was examined whether the extract promoted its anti-inflammatory effect through a glucocorticoid mechanism.

Glucocorticoids have various functions, such as anti-inflammatory, antimitotic, apoptosis-inducing, vasoconstrictive and immunomodulatory functions. They act in two different ways at the cellular level, either by the genomic or non-genomic pathways. The genomic pathway begins with binding to the glucocorticoid receptor (GR), causing receptor homodimerization, and then the dimerized receptor binds to glucocorticoid responsive elements, and migrates to the nucleus. The dimerized GR binds to a palindromic promoter sequence and promotes transcription of genes with anti-inflammatory functions, such as
tyrosine amino transferase, phosphoenolpyruvate carboxykinase, and IL-10. The complex also negatively regulates the expression of pro-inflammatory cytokine genes, growth factors, and adhesion molecules, among others, by transrepression of NF-κB. The non-genomic pathway is responsible for the rapid effects of glucocorticoids and is mediated by membrane linked receptors and second messengers. This pathway does not require synthesis of new proteins and acts by modulating the level of activation and responsiveness of target cells, such as monocytes, T-cells and platelets (Uva et al., 2012).

It is an established fact that dexamethasone promotes its anti-inflammatory effect by interacting with GRs, and here this fact was once more observed since the animals pretreated with mifepristone exhibited a significant reversal in the dexamethasone-mediated inhibition of edema formation and in MPO activity. The results also showed that EHSG may be acting partially by interaction with GRs since its inhibitory effect over MPO was totally reversed by mifepristone, but not its effect on edema. The possible interaction of EHSG and GRs was demonstrated using the specific binding of [1H]-dexamethasone and confirmed that EHSG is able to reduce binding of dexamethasone to the GR at high concentrations.

Skin atrophy is one important side effect of topical glucocorticoid therapy and is characterized by a marked increase in skin transparency, and is accompanied by an increased fragility, purple spots, and telangiectasia (Schoepe et al., 2006). It was observed that dexamethasone promoted atrophy in the ears and a change in behavior of mice as a result of repeated applications. Nevertheless, animals that received EHSG showed no changes in ear thickness, behavior, body weight or blood glucose. In contrast, repeated application of the extract interfered with thymus, spleen, adrenal gland and lymph nodes weight, just as dexamethasone did. This result shows that the EHSG is probably being absorbed and reaching the systemic route, generating these side effects similar to corticoids. This observation reinforces its hypothesized mechanism of action via the GR pathway. In the genotoxicity test, both EHSG and dexamethasone did not promote DNA damage, and actually the indexes were lower than the vehicle group, indicating that the treatments prevented cells from undergoing chromosomal mutations. Thus, it is possible that EHSG is acting partially through the GR pathway, but with fewer side effects when applied topically.

Conclusion

From these initial results, EHSG was demonstrated to be a potential tool for the treatment of inflammatory skin diseases, as evidenced by its topical and systemic anti-inflammatory activity, and reduction in inflammation parameters such as edema formation, leukocyte migration, and pro-inflammatory cytokine levels (IL-1β, IL-6 and TNF-α) in models of acute and chronic inflammation. This anti-inflammatory effect of EHSG can be, at least partly, attributed to an activation of the GR pathway. However, other parameters need to be evaluated to better elucidate the mechanism of action for EHSG. In addition, a complete phytochemical analysis of the extract is required in order to identify the compounds present and further evaluate the toxic potential of the plant.
Fig. 8. Effect of adverse of multiple topical applications of EHSG and dexamethasone on lymphoid organs. (A) Representative pictures of organs removed, (B) thymus, (C) spleen, (D) adrenal gland and (E) auricular lymph nodes. ∗∗P < 0.01 and ∗∗∗P < 0.001 compared to vehicle group.
Conflict of interest

The authors declare that they have no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phymed.2016.10.003.

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