WITHDRAWN: *Sambucus Ebulus* Fruits Extract Ameliorates Eosinophilic Nasal Polyposis: An in vitro Study

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EDITORIAL NOTE:

The full text of this preprint has been withdrawn by the authors while they make corrections to the work. Therefore, the authors do not wish this work to be cited as a reference. Questions should be directed to the corresponding author.

Abstract

Background: The *Sambucus Ebulus (ES) L*. has an anti-inflammatory role by reducing the expression of pro-inflammatory genes and free radicals. The aim of this study was to evaluate the anti-inflammatory effect of hydroalcoholic extract of SE fruits on eosinophilic nasal polyposis (NP) in Vitro.

Method: The chemical composition of SE fruits extract was determined by gas chromatography-mass spectrometry. Polyp Samples were collected from 40 patients and were cut into four pieces (control and case groups). Tissue fragments were treated with 0 (control), 50, 315 and 1000µg/ml of SE FRUIT extract in a culture medium for 24 hours. Apoptosis was detected by TUNEL reaction reagent. Real time-PCR was employed to evaluate the expression of Bax and Bad genes. ELISA was used for assay of IL-5 and GM-CSF.

Results: Our findings showed SE fruits extract increased apoptosis in nasal polyp tissues compared to the control group ($P<0.001$). Also, GM-CSF level in the experimental groups was significantly lower than that in the control group ($P<0.001$). In contrast, the level of IL-5 was not significantly different in the treatment and control groups ($P>0.05$). Bax and Bad expression showed a significant increase in the experimental groups compared to the control group ($P<0.001$).

Conclusion: Our findings showed that SE fruits hydroalcoholic extract ameliorates NP trough elevation of apoptosis and reduction of inflammatory markers. Our experimental data revealed that SE fruits extract plays an important role in the treatment of nasal polyp through inducing apoptosis and reducing the survival of inflammatory cells.

Background

Nasal polyposis (NP) is one of the most common forms of chronic rhinosinusitis with a high recurrence rate [1]. It is characterized by progression of an inflammatory mass into the nasal cavity, leading to complications such as nasal obstruction, reduce sense of smell, headache, and consequently reduce quality of life [2, 3, 4]. NP as a multifactorial disease is usually associated with diseases such as asthma and other respiratory diseases, including cystic fibrosis and aspirin sensitivity [3, 5]. It is reported that, the prevalence of NP in various populations is 1–4% [6].

Eosinophilic NP is the most common type of NP. Approximately 65–90% of all NP occurs by infiltration of eosinophils into polyp tissues during inflammatory processes [7]. Eosinophils and other cells are located in NP produce cytokines that sustaining the inflammatory process and invoking new eosinophils, which in turn result in tissue damage. Therefore, eosinophils are should be removed from polyp tissues by apoptosis to inhibit the inflammation process [8]. It is noteworthy that, the apoptosis rate of eosinophils in polyp tissues is less than the nasal mucus of healthy people.

The β common cytokine family such as interleukin-5 (IL)-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) result in the survival of these cells via reducing the eosinophilic apoptotic

index [8, 9]. Besides, GM-CSF plays critical roles as a resolution of inflammatory responses and in the development of chronic inflammation [10]. Therefore, it seems that therapeutic strategies that decrease and inhibit inflammation would be an appropriate approach. Corticosteroids are prescribed as the most common medicine in clinics to reduce the inflammatory process in sinonasal polyposis [11, 12]. Steroids, like many other pharmacological agents, are not completely safe and have complications on cardiac, neurological, musculoskeletal, as well as digestive system [13]. Therefore, it is necessary to find an appropriate drug with minimal side effects.

Herbal medicines have been traditionally used in different communities. Of noted, the long-standing position of herbal medicines in public health has attracted scientific and research centers in the last century. Sambucus Ebulus (SE), belonging to the Caprifoliaceae family, is a perennial herb with green leaves that appears in the late spring with white or creamy flowers [14]. In traditional Iranian medicine, it is used topically to treat inflammations caused by insect bites and also some herbs such as nettle [15, 16]. It has been revealed that leaf extract of SE had an inhibitory effect on inflammatory cytokines IL-1 (IL-1α & IL-1) and TNF-α [17]. Moreover, other studies revealed that SE contains several compounds, including phenolic acids, flavonoids, steroid, vitamin C, caffeic acid, anthocyanins and cyanogen lycosides [18, 19]. In another study, it has been shown that SE hexanic extract from fruits and leaves in the northern part of Iran has the most anti-inflammatory effect [16]. In another study, it has been shown that SE hexanic extract from fruits and leaves in the northern part of Iran has the most anti-inflammatory effect [18, 20].

Therefore, due to the presence of various beneficial components in this plant and its possible effect on reducing the inflammatory process, the purpose of the present study is to determine the therapeutic effects of the hydroalcoholic *SE* fruit extract on preventing and treating of NP and its possible role in apoptosis and inflammation.

Materials & Methods

SE fruits extraction

SE fruits were collected in Guilan (a province in the north of Iran: with herbarium number, 301HGUM) dried at room temperature and were kept away from direct sunlight. SEF (500g) were soaked in 6 liters of ethanol 70% to prepare a hydroalcoholic extract. Concentration and drying of the extracts were performed after centrifugation using a rotary vacuum evaporator. Then, the extract was transferred to an oven and completely dried at 40°C. The dried extract was weighted and dissolved in Phosphate-buffered saline (PBS) under laminar hood and sterilized using a 0.22μm filter. Our working solution was prepared by dilute DMEM-F12 for the required doses (X1 = 50, X2 = 315, and X3 = 1000 μg / ml).

Separation and identification of SE fruits by gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS conditions

All analysis was carried out on a GC-MS system from Agilent Technologies (Wilmington, DE, U.S.A.) GC-MS system has been used in this study consist of a model 7890A gas chromatograph, a model 5970B mass selective detector and electron ionization (EI) mode (70 eV). A fused-silica capillary column (30 m × 250 m i.d., 0.25 m film thickness) from Scitech Scientific was also used. The temperature program of GC-MS was as follows: initial temperature was 120 °C, held for 2 min, increased to 140 °C at a rate of 2 °C/min, then to 220 °C at a rate of 3 °C/min, and finally to 320 °C at a rate of 7 °C/min and held for 15 min. The split ratio was 1:12, injection temperature was 290 °C, transfer line temperature was 310 °C, and ion source temperature was 240 °C. A quadrupole mass spectrometer (MS 5975 C inrtXL EI/CI MSD with triple-axis detector) was operated at 70 eV in the electron impact CTC PAL auto sampler.

NP' tissue culture

NP samples were obtained from 40 subjects referring to the hospital affiliated with Guilan University of Medical Sciences in the north of Iran. The present study was approved by the Ethics Committee of Guilan University of Medical Sciences (Number: IRGUMS.REC.1395.311) and all the participants completed the informed consent after the purposes of the study were explained to them. NPs were diagnosed based on the criteria for chronic rhinosinusitis with NP. The patients with non-eosinophilic polyposis, eosinophilia, and infectious processes, and the patients consuming corticosteroids less than 4 weeks before surgery were excluded.

NP tissues with ≥40% eosinophils which were confirmed by Hematoxylin and Eosin (H&E) (Fig.1) were considered in the study. The samples were washed 3 times with 10% PBS solution supplemented with 1% penicillin-streptomycin antibiotics and antimycotic (antifungal). Then, the specimens were carefully cut with a sterile blade under a microscope in sterile condition. 4 pieces (approximately 3 mm^3) were selected for TUNEL tests from the NP tissue samples of each patient. Other remaining specimens were cut into smaller pieces (approximately 1mm^3) and prepared for culture for genes and proteins analyses. The NP tissue was cultured in DMEM-F12 supplemented with 1% penicillin-stereptomycin antibiotics, 10%FBS and various doses of SE fruits extract in 6well plate in 37º C with 5% Co2. The cells were treated with 0 (as a control), 50, 315 and 1000µg/ml [21] of SE fruits extract for 24 hours.

Apoptosis detection with Terminal deoxyribonucleotidyl transferase-mediated dUTP nick end labeling (TUNEL)

Apoptosis was detected using in situ cell death detection kit (Roche, Germany) in NP tissues according to company manual. Briefly, the 5µm sections were dewaxed and rehydrated according to the standard protocols (by heating at 60˚C, rising in xylene for 10 minutes and then rehydration through a graded series ethanol and ddH2O). Afterward, the slides were washed with PBS and incubated with Proteinase K for 20 minutes at 37 ° C. The specimens were incubated for 10 minutes with a permeablization solution and rinsed again with PBS. In the next step, 50µl of TUNEL reaction solution was poured onto each tissue sample and incubated for one hour at 37 ° C, and after the final washing, they were observed with a Zeiss LSM 5 fluorescent microscope. Apoptotic positive cells were counted at least in 5 fields in each group. A

surface of 1-2 mm in each group was considered for counting. In this protocol, TUNEL-Enzyme solution and TUNEL-Label solution were used to color the positive control samples from 1 to 9, while for negative control samples; only TUNEL-label solution was used. Apoptotic cells in this tissue were bright spots indicating apoptotic cells marked during TUNEL. The nucleus of the cells was identified as blue with 4 ', 6-diamidino-2-phenylindole (DAPI). 4 ', 6-diamidino-2-phenylindole (DAPI) was applied as a counterstaining and the nucleus represent a blue color. The percentage of apoptotic cells was determined by dividing the number of apoptotic cells on the total number of cells.

Real-Time PCR for expression of Bax and Bad

Total RNA was extracted from cells using the InnetiPREP RNA mini kit (Analytikiena, Germany) according to company protocol. The contaminating DNA from RNA preparation was removed using Sinacolon (Iran) kit according to the manufacturer's protocol. cDNA was synthesized using BioFACT(BioFACT, Korea) kit according to the manufacturer's instructions. Real-Time PCR was performed using SYBR-Green methods through the Step One Plus™ real-time PCR instrument to measure the quantitative expression of Bax and Bad genes in different groups. The Ct value was calculated by one step software (Applied Biosystems) using the automatic threshold setting. The relative expression of Bax and Bad relative to the reference gene of GAPDH was performed in triplicates using the ΔΔCT method. The intended genes were amplified with primers shown in Table 1.

Table 1.
Primer sequences for *Bax*, *Bad* and *GAPDH*.

Primer		Sequences (5' to 3')
GAPDH	FORWARD	ACCCACTCCTCCACCTTTGA
	REVERSE	CTGTTGCTGTAGCCAAATTCGT
Bax	FORWARD	GTCGCCCTTTTCTACTTTGCC
	REVERSE	CTCCCGCCACAAAGATGGTCA
Bad	FORWARD	CCAGATCCCAGAGTTTGAGCC
	REVERSE	CCATCCCTTCGTCGTCCTCC

Enzyme-linked immunosorbent assay (ELISA) for evaluation of the level of IL-5 and GM-CSF

To evaluate the level of IL-5 and GM-CSF, the NP was cultured with 0, 50, 315 and 1000µg/ml of SE extract for 24h. Then, the culture medium in which the NP was cultured collected and stored at -80°C for future analysis. To evaluate the level of IL-5, Estbiopharm (USA), a human IL-5 kit was used. Besides, the level of GM-CSF was detected by Human GM-CSF ELISA MAXTM kit Deluxe Set (Biolegend, USA). The optical density of the specimens was recorded. The standard curve was used to determine concentration of unknown samples.

Statistical analysis

All data were presented as mean ± Standard deviation (SD). The data were analyzed by Statistical Package for the Social Sciences (SPSS) software Version 16. First, the data were normalized through the One-Sample Kolmogorov-Smirnov test, and after confirming the normality of the data, they were analyzed by one-way Analysis of variance (ANOVA). The significance level was considered as $P<0.05$.

Results

Gas chromatography-mass spectrometry analysis

Major chemical composition of SE fruits extracts is shown in Table 2. The main constituents with medical and therapeutic properties of this plant were phenolic compounds that are found in great numbers in the fruits of this plant, which are associated with organoleptic, nutritional, and antioxidant properties.

Table 2
Compounds Contained in the methanolic Extract of Sambucus Ebulus fruits.

Compound	Percent
Caffeic acid [a]	5.2
Ascorbic acid	1.1
p-Hydroxybenzoic acid	0.2
Palmitinic acid	3.2
Acetic acid	0.3
Octadecadienoic acid	0.2
Ethyl acetate	0.4
Oleic acid	11.3
2-Methoxy-4-vinyl Phenol	0.6
4-Ethyl catechol	0.1
Octadecanoic acid	0.2
Dehydroabietic acid	0.4
Benzoic acid	0.7
Campesterol	0.8
1,2-Benzenediol	0.7
Furfual	0.6
Stigmasterol	0.2
3-Methylbutanoic acid	0.2
β-Sitosterol [a]	10.7
2-Ethoxy butane	0.5
n-Hexadecanoic acid	1.2
Benzene acetic acid	1.8
Oleanolic acid	0.7
4-Ethyl-1,3-benzenediol	0.9
Ursolic acid [a]	18.8
2-methoxy-4-vinyl phenol	0.3
	2.3

[a] The component that may have therapeutic effects on nasal polyposis

Compound	Percent
1,1-Diethoxyethane	0.7
Maslinic acid	13.7
9,12-Octadecadienoic Acid [a]	22
Other	

[a] The component that may have therapeutic effects on nasal polyposis

SE fruits extract increased apoptosis in NP

Immunofluorescence TUNEL assay was investigated to detect apoptosis following treatment of SE fruits extracts on NP tissues. Figure 2 shows the presence of the fragmented genomic DNA indicates the formation of apoptotic bodies and the apoptotic nucleus is seen in light green (Fig. 2). Our data showed that apoptosis of eosinophils significantly increased in the groups that received different doses of the SE fruits extract compare to control group ($P<0.0001$). Furthermore, the induction of apoptosis pattern was dose-dependent and significantly was higher at 1000 µg/ml of SE extract ($P=0.004$) (Fig. 3A).

SE fruits extract increased expression of anti-inflammatory factors

To confirm the anti-inflammatory effect of SE FRUIT extract, real-time PCR was performed for gene expression analysis. High expression of Bax indicated anti-inflammatory effect after treatment with 50, 315, and 1000 µg/ml of the SE FRUIT extract for 24 hours. The Bax expression in different doses of SE FRUIT extract was significantly higher than that in the control group ($P<0.0001$) (Fig. 3B). In addition, expression of Bad increased in NP after treatment with different doses of the SE FRUIT extract ($P<0.0001$). A comparison of Bad gene expression between different treatments demonstrated a significant relationship between low and high doses ($P=0.033$) (Fig. 3C).

SE fruits extract decreased inflammatory cytokine in NP

Evaluation of NP culture medium after treatment with SE fruits extract with ELISA showed that the level of IL-5 was not significantly different in the treatment and control groups ($P>0.05$) (Fig. 3D). However, the level of GM-CSF was significantly decreased in groups that received different doses of the SE FRUIT extract compare to control groups ($P<0.0001$). Although GM-CSF levels increased synchronically with the groups receiving the higher dose, this increase was not statistically significant (Fig. 3E).

Discussion

This study showed that the hydroalcoholic extract of SS fruit has anti-inflammatory properties on nasal polyp tissue. Increasing GM-CSF improves the inflammatory conditions caused by nasal polyps. On the

other hand, our findings showed that the percentage of apoptotic cells compared to control groups in nasal polyp tissues treated with different doses of SE FRUIT extract increased significantly.

NP disease is recognized as one of the most common cases of chronic rhinosinusitis [1, 2]. Currently, functional endoscopic sinus surgery is used for treatment of advanced stages of disease and steroid anti-inflammatory drugs are used for control and treatment at early stages. Surgical treatment is an invasive and high-risk process, and steroids, like many chemical drugs, have various side effects [12, 14]. Although medicinal plants have been introduced as a promising strategy in treatment of some diseases, its therapeutic properties are not fully understood. The current study, we analyzed the effects of SEL. (that is a popular and available herbaceous plant in folk medicine in Iran) on Bad and Bax gene expression, level of IL-5 and GM-CSF cytokines after 24 h exposure to SE FRUIT. Our study specified that using SE FRUIT increase apoptosis in polyp tissue and raise levels of anti-inflammatory cytokines.

It is suggested that multiple mechanisms appear to be involved in increasing inflammation. First, GM-CSF participates in the resolution of inflammation, and IL-3 and IL-5 may also have such properties [10]. In addition, It has been reported that GM-CSF and IL-5 stimulation increase eosinophil gene expression [22]. Similar to IL-5, another cytokines including IL-3 and GM-CSF cytokines are produced as part of the Th2 inflammatory response and are crucial to eosinophil development and function. Second, the physiological effects of each of these cytokines are mainly controlled by the expression of the cytokine-specific receptor, which are highly impact on target cells. Third, lymphoid cells produce GM-CSF during inflammation process and amount of systemic GM-CSF increase dramatically in circulation during inflammation process [23]. We found that SE FRUIT extract reduced the amount of GM-CSF significantly compare to control group ($P < 0.0001$) and exerts its anti-inflammation property through GM-CSF inhibition.

Our results indicated that SE FRUIT extract increased the level of IL-5 in 50 and 315 µg in vitro, however, the SEs fruits extract decreased the level of IL-5 at 1000 µg/mg SE FRUIT extract in the NP tissues, but it was not significant ($P > 0.05$). Recently, some studies focused to decrease IL-5 in NP, which is only βc cytokine that have an essential role in differentiation, and proliferation of eosinophils [24]. Van Zele et al. showed that methylprednisolone as a chemical drug exhibited therapeutic effects on NP by decreasing IL-5, eosinophil cationic protein and immunoglobulin E [25], being consistent with our findings in reducing IL-5 in high doses of the extract. Some studies revealed that low doses of chemical drugs could not induce their therapeutic potency on NP tissues. Besides, allergic diseases that are diagnosed by tissue infiltration of eosinophils can promote long-term tissue injury and fibrosis [26]. Therefore, researchers used the therapeutic strategy such as inhibition of IL-5, and they showed that this can improve esophageal inflammation in children [27].

Our findings decipher that level of GM-CSF, another key cytokine, significantly decreased after treatment of NP with SE fruits extract. Many studies have demonstrated the importance of GM-CSF and its significant role in the development of NP. Therefore, one of the main goals of researchers is decreasing GM-CSF level and in turn decreases the complications of NP. Taken together, our results indicated that

hydroalcoholic extract of SE FRUIT has a significant anti-inflammatory effect on NP through suppression of IL-5 and GM-CSF cytokines. Of noted, SE fruits could suppress GM-CSF factor more effectively rather than IL-5, however, at the high dose the results are similar. Since, the cytokines are directly association with inflammatory cells; their decline can be justified with anti-inflammatory property of SE FRUIT extracts on inflammatory cells, particularly eosinophil.

On the other hands, several studies have indicated that induction of apoptosis in inflammatory cells of the NP, particularly eosinophils, can play a significant role in improving the disease conditions [21, 28]. The results of the GC-MS technique in this study showed that 20.9% of the metanolic extract of SEs contained a natural pentacyclic trisperpenic acid (ursolic acid). Ursolic acid is commonly found in various parts of medicinal plants including leaves, flowers, and fruits and has proven properties such as anti-inflammatory, antioxidant and anti-carcinogenic properties [29]. Ursolic acid has been reported to inhibit the inflammatory factors TNF-α and IL6, LOX-1, VCAM-1, ICAM, IL5, IL13 and IL17 [30]. Another study shows that ursolic acid increases apoptosis in lung cancer cells [31]. In addition, it is also reported that UA via decreasing Bcl-2, Mcl-1, TCTP expression and increasing apoptotic proteins such as TNF-α, Fas, FADD and BAX results in activation of caspase-3 and PARP which induces apoptosis of the liver cancer cells. Therefore, the increase of apoptosis and the decrease of inflammatory factors in this study are probably due to the effect of this substance. Our results of apoptosis detection showed that the percentage of apoptotic cells significantly increased in comparison to the control group in the NP tissues treated with different doses of the SE FRUIT extract in dose dependently. It is suggested that the formation of apoptotic bodies probably inhibit the release of intracellular contents and inactivated inflammatory mediators, therefore exerts its anti-inflammatory property[32].

Furthermore, high expression of pro-apoptotic genes Bax and Bad confirmed the results obtained from in situ cell death detection with TUNEL. It has been revealed that, the imbalance between Bcl2 and Bax gene expression causes caspase9 activation and leads to apoptosis, indeed, direct activation of caspase3 is another way for causing apoptosis[33]. Our findings decipher that SE fruits extract increased significantly two pro-apoptotic key genes Bax and Bad in NP tissues ($P < 0.0001$). On the other hand, Beta-sitosterol, one of major component of SEs extract (Table2) has a crucial role in inducing apoptosis in NP tissue[34]. Researchers have been reported Beta-sitosterol as an inducer of apoptosis through the activation of P53 gene and induction of cell cycle arrests may have contributed to increased apoptosis in NP tissue after receiving SEs fruit extracts. Several investigations on drug treatment of NP have shown that inducing transduction of pro-apoptotic genes increases the expression of these genes in NP, leading to apoptosis induction in inflammatory cells, and as a result, the treatment of NP [35].

Conclusion

Our results demonstrated that hydroalcoholic extract of SE fruits reduced the process of inflammation of the NP by inducing apoptosis of inflammatory cells such as eosinophil as well as by reducing inflammatory cytokines in dose-dependently. Thus, hydroalcoholic extract of SE fruits may play beneficial and significant roles in the treatment of patients with eosinophilic NP at the early stages of the disease

and could be possibly used in the clinical applications after comprehensive studies. We suggest, the presence of diverse and effective compounds in SEs fruits could provide the opportunity for further comprehensive researches in the field of anti-inflammatory mechanisms and induction of apoptosis in vitro and in vivo studies.

Abbreviations

Sambucus Ebulus: SE

Nasal polyposis: NP

Granulocyte-macrophage colony-stimulating factor: GM-CSF

Interleukin: IL

Tumor necrosis factor - α: TNF-α

Phosphate-buffered saline: PBS

Gas chromatography-mass spectrometry: GC-MS

Electron ionization: EI

Hematoxylin and Eosin: H&E

Terminal deoxynucleotidyl transferase dUTP nick end labeling: TUNEL

Polymerase chain reaction: PCR

Cycle threshold: Ct

BCL2 Associated X: Bax

BCL2 Associated Agonist of Cell Death: BAD

Glyceraldehyde-3-Phosphate Dehydrogenase: GAPDH

Enzyme-linked immunosorbent assay: ELISA

Analysis of variance: ANOVA

Standard deviation: SD

Statistical Package for the Social Sciences: SPSS

B-cell lymphoma 2: Bcl-2

Declarations

Ethics approval, consent to participate &Funding

This study is extracted from an anatomical Science MSc student dissertation (registration number-151). This dissertation was approved by the Ethics Committee of Guilan University of Medical Sciences (Number: IRGUMS.REC.1395.311) and all the participants completed the informed consent.

Availability of data and materials

The data is available after the reasonable request from the responsible author.

Competing interests

The authors declare that there was no conflict of Interest during performing this study.

Authors' contributions

M.F: Conceptualization, Writing- Original draft preparation - Reviewing and Editing

B.P: Investigation, Writing- Original draft preparation - Reviewing and Editing

M.A: Investigation, draft preparation, Reviewing and Editing

A.Z: Investigation, Reviewing and Editing

S.N: Sample collection, Reviewing and Editing

S.R: Investigation, Reviewing and Editing

F.Y: Investigation, Reviewing and Editing

H.B: Sample collection, Reviewing and Editing

M.G: Investigation, draft preparation, Reviewing and Editing

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Figures

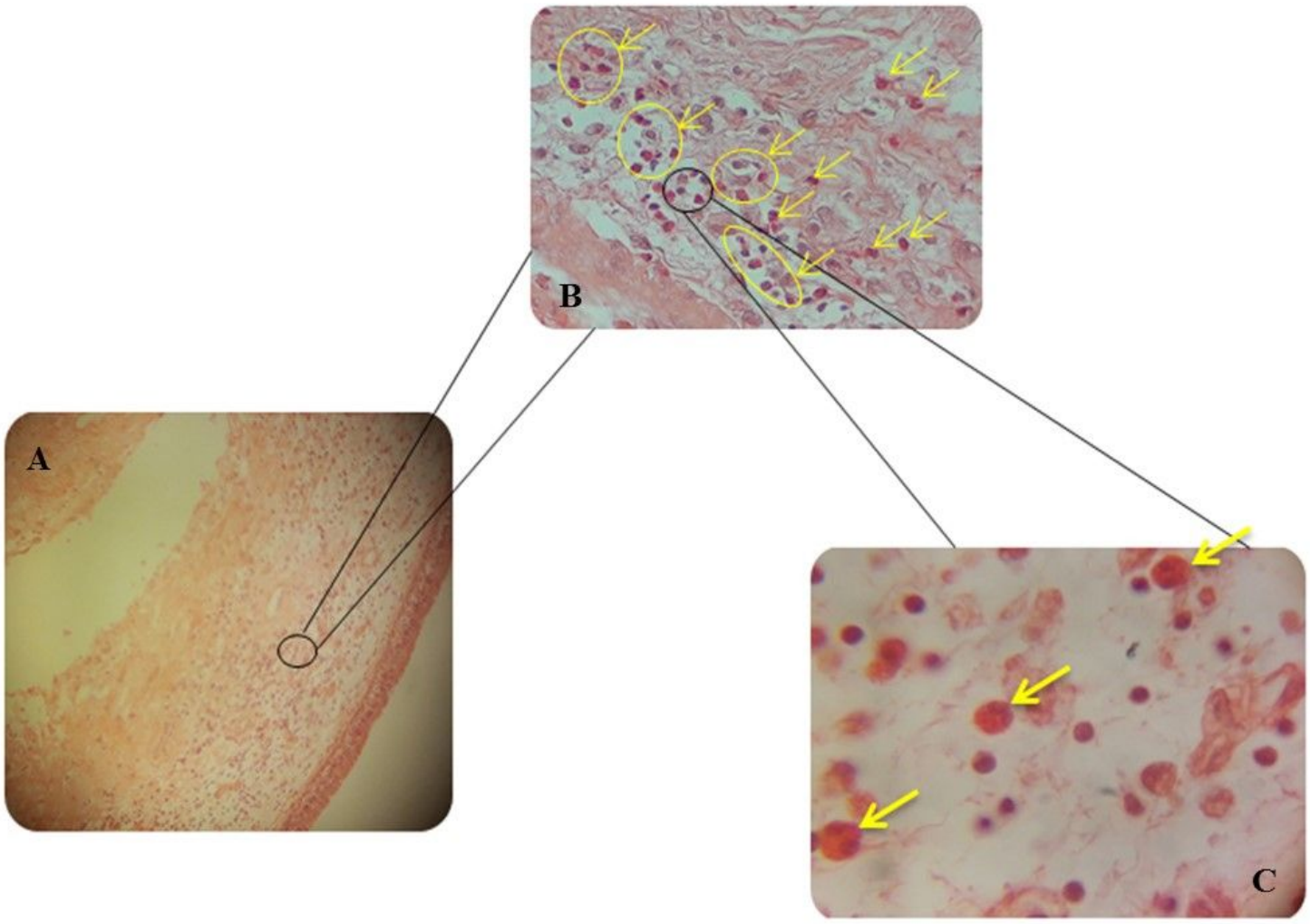

Figure 1

Eosinophils dispersion in eosinophilic nasal polyposis. A: ×10. B: ×40. C: ×100. H&E Staining. Eosinophils are marked with yellow arrows.

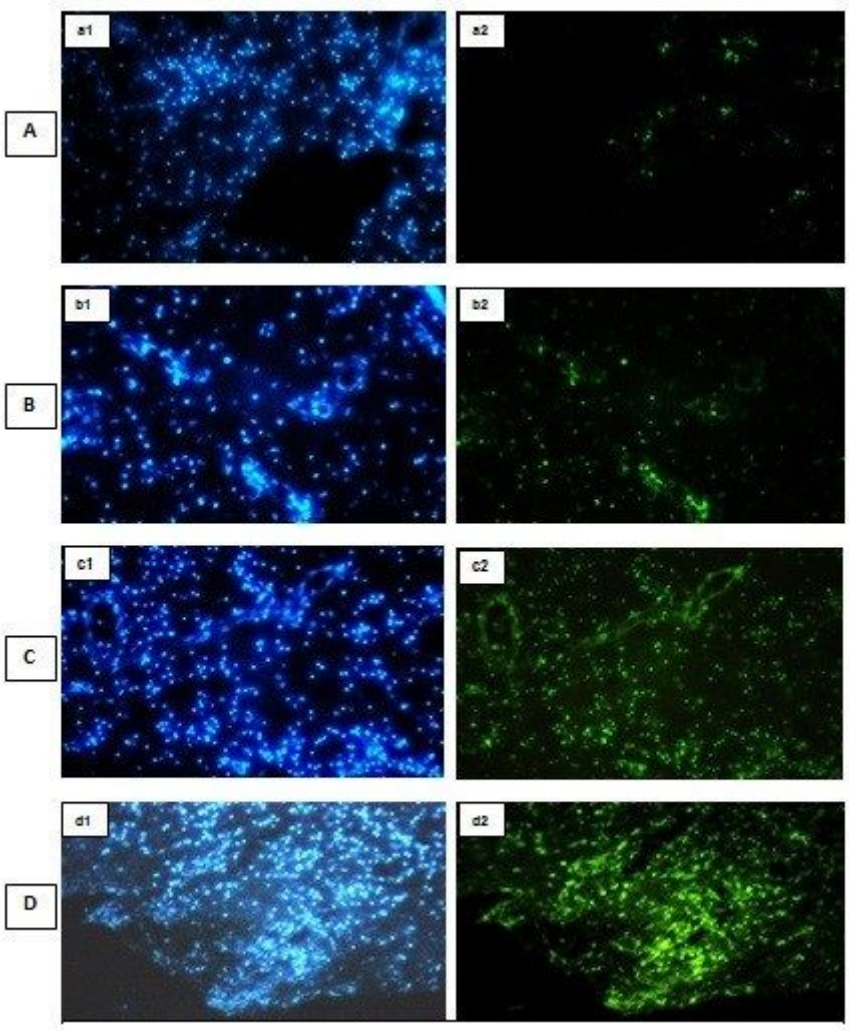

Figure 2

Detection of apoptosis by TUNEL assay in nasal polyp tissues was controlled and treated with different doses of SE fruits extract. Control group(A): a1 is related to the 4', 6-diamidino-2-phenylindole(DAPI) and indicates all nuclei in bright blue. a2 represents apoptosis where apoptotic nuclei are indicated in bright green. The group under treatment with a dose of 50 µg/ml(B): b1 is related to the DAPI and indicates all nuclei in bright blue. b2 represents apoptosis where apoptotic nuclei are indicated in bright green. The group under treatment with a dose of 315 µg/ml(C): c1 is related to the DAPI and indicates all nuclei in bright blue. c2 represents apoptosis where apoptotic nuclei are indicated in bright green. The group under treatment with a dose of 1000 µg/ml(D): d1 is related to the DAPI and indicated all nuclei in bright blue. d2 represents apoptosis where apoptotic nuclei are indicated in bright green.

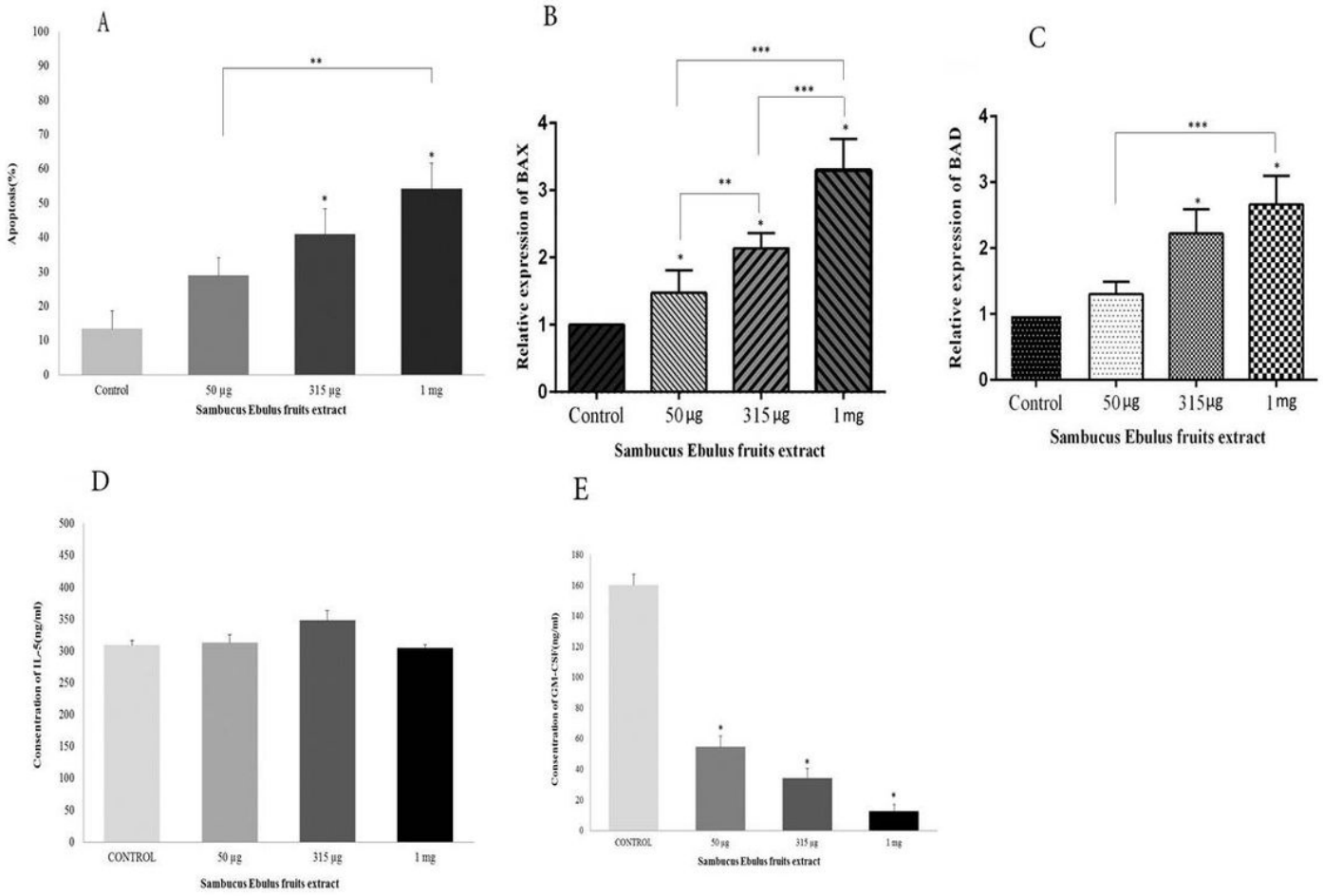

Figure 3

A: Results of the percentage of apoptosis in nasal polyp tissues on day1. The percentages of apoptosis in nasal polyp tissues were evaluated by TUNEL assay. The amount of apoptosis was elevated with dose increase. * p<0.05 vs. 315 µg/ml SE extract group and control group. * p<0.05 vs. 1mg/ml SE extract group and control group. **P<0.001 vs. 1mg/ml SE and 50 µg/ml SE extract groups. B&C: Results of Real Time PCR for BAX and BAD expressions in polyp tissue on day2 relative to GAPDH gene by using the ΔΔCT method. Values are the means ±SD. (B)*P<0.05 vs. SE extract groups and control group. ** p<0.005 vs. 50 µg/ml and 315 µg/ml SE extract groups. ***P<0.001 vs. 1mg/ml and 315 µg/ml SE extract groups. ***P<0.001 vs. 1mg/ml SE and 50 µg/ml SE extract groups. (C)*P<0.05 vs. 1mg/ml and 315 µg/ml SE extract groups and control group. ***P<0.001 vs. 1mg/ml and 50 µg/ml SE extract groups. D&E: The results of ELISA analysis for Concentration of IL-5(A) and GM-CSF (B) in polyp tissue with and without SE on day2. Values are the means ±SD. (D) no significant difference between SE extract groups and control group. (E)*P<0.05 vs. SE extract groups and control group. SE: Sambucus Ebulus