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Anti Inflammatory Effect of Grape Seed (*Vitis vinifera*) Extract on Formalin-Induced Edema in Rat Paw

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Abstract: Several studies have shown that grape seed extract possess anti-inflammatory effect because of its components such as procyanidine. The purpose of this study was the investigation of the anti-inflammatory effect of grape seed extract on formalin-induced edema in rat paw. Hydroalcohlic extract of grape seed (*Vitis vinifera*) was prepared using maceration method. Albino rats weighting 180-200 g were divided in to 5 groups as intraperitoneal GSE receiving (200, 400 and 600 mg kg^{-1}), positive control (indomethacin 5 mg kg^{-1} p.o.) and negative control (normal saline 5 mg kg^{-1} IP), 30 min following IP injection of GSE or indomethacin administration 0.1 mL of formalin (2.5% w/v) was injected in rat hind paw and the changes in rat's paw volume was assessed by plethysmometer for 3 h at intervals of 1 h. Grape seed extract (200, 400 and 600 mg kg^{-1}, IP) exerted anti-inflammatory effect in a dose dependent manner. Hydroalcoholic seed extract in dose of 600 mg kg^{-1} was more effective against formalin-induced paw inflammation. However, this effect was less potent than indomethacin (5 mg kg^{-1}) effect as positive control.

Key words: Grape seed, inflammation, rat paw edema, *Vitis vinifera*, injection, Iran

INTRODUCTION

Inflammation is a basic way in which the body reacts to infection, irritation or other injury, the key feature being redness, warmth, swelling and pain. Inflammation is now recognized as a type of nonspecific immune response (Ferrero-Miliani *et al*., 2007; Kumar and Collins, 1998).

Often the inflammatory response is out of proportion to the threat, it is dealing with the result can be more damage to the body than the agent itself would have produced. Many types of allergies and most of the autoimmune diseases are examples of inflammation in response to what should have been a harmless or at least noninfectious agent (Janeway and Travers, 1996). Therapeutic agents that used in inflammatory diseases such as NSAIDs (Non-steroid Anti Inflammatory Drugs), glucocorticoids and immunosuppressive drugs have a high level of side effects and introducing a new anti-inflammatory agent with low levels of adverse effects can help patients with inflammatory diseases.

Functional foods and nutraceuticals have been targeted to promote health and well-being in an array of disease states. They are receiving widespread interest in terms of prevention of inflammation and other disease. Their relative safety and lower adverse effects, cost as well as the relative ease of access by the general population via a range of food products and supplements make a sound case for discovering novel bioactives with increased efficacy (Ortega, 2006; Kris-Etherton *et al*., 2002). The medicinal and nutritional value of grapes (*Vitis vinifera*) has been heralded for thousands of years. Egyptians consumed this fruit at least 6,000 years ago and several ancient Greek philosophers praised the healing power of grapes. European folk healers developed an ointment from the sap of grape vines to cure skin and eye diseases. Among other beneficial effects, the active compounds in grape seed are believed to have antioxidant properties (Busserolles *et al*., 2006). In fact, a recent study of healthy volunteers found that supplementation with grape seed extract substantially increased levels of antioxidants in the blood (Vitseva *et al*., 2005).

Vitamin E, flavonoids, linoleic acid and compounds called procyanidins (also known as condensed tannins, pycnogenols and Oligomeric Proanthocyanidins or OPCs are highly concentrated in grape seeds (Vigna *et al*., 2003; Pataki *et al*., 2002). Grape seed and red wine extracts are known to contain high levels of polyphenolic compounds and consumption of these compounds have been attributed to their beneficial health effects (Freedman *et al*., 2001; Laurent *et al*., 2004). Phenolic compounds of grape seeds may help to inhibit enzyme systems that are responsible for the production of free radicals and that are associated with inflammatory reactions. Procyanidins intervene in the synthesis and

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release of many substances that promote inflammation for example, histamine, serine protease, prostaglandins and leukotrienes (Shi *et al*., 2003; Hemmati *et al*., 2006). Recently, many studies have been performed on the anti-inflammatory effect of grape seed, e.g., Hemmati *et al*. (2008) showed the protective effect of grape seed extract against the fibrogenic effect of bleomycin or silica in rat lung.

In other studies, it was indicated that Grape Seed Proanthocyanidin Extract (GSPE) reduces the expression of IL-6 (Interleukin- 6) and MCP-1 (Monocyte Chemotactic Protein-1) and enhances the production of the anti-inflammatory adipokine adiponectin suggest that GSPE may have a beneficial effect on low-grade inflammatory diseases such obesity and type 2 diabetes (Chacon *et al*., 2009). Results of another experiment indicated that intraperitoneal injection of GSE attenuated CIA (Collagen-induced Arthritis) in mice and suggest that GSPE may be useful in the treatment of rheumatoid arthritis (Terra *et al*., 2009). On the basis of these common uses of grape products in traditional folk medicine and its recently reported activities in literature, we have investigated the anti-inflammatory effect of grape (*Vitis vinifera*) seed extract using rat paw edema model that no such investigation has been reported.

MATERIALS AND METHODS

Animals: Adult male albino rats (weighting 180-200 g) obtained from Animal House and Research Center, Jundishapur University of Medical Sciences, Ahwaz, Iran were used for the experiment. The animals were maintained in an environment with controlled temperature (22± 2°C) with a relative humidity of 50-75%. The animal room was on a 12 h photo-period cycle. Food and water were provided *ad libitum*.

Plant material: Grape seeds were a gift from Sasan Shahd Dietary Industries (Urommia, Iran). Seeds were obtained from red grapes (*V. vinifera*) in the process of grape juice production. Grape seeds were dried in drying oven at 50°C for 72 h. Then dried seeds were ground to fine powder by a grinder. About 200 g of the powder was mixed with 600 mL of 70% ethanol in distilled water and kept for 3 days at room temperature. The extract was then filtered through a buchner funnel. Solvent (ethanol/water) was removed using rotary evaporator under vacume at 50°C. The residue (dried extract) obtained and kept in refrigerator for further experiments.

Formalin and indomethacin: Formalin purchased from merck and indomethacin powder was a gift from Amin Pharmaceutical Co. (Isfahan, Iran).

Experimental groups: Thirty animals were randomly divided into the five groups as fallows:

- **Group I:** Indomethacin 5 mg kg^{-1} was administered po (positive control)
- **Group II:** Normal saline 5 mg kg^{-1} was administered IP (negative control)
- **Group III:** GSE (200 mg kg^{-1} IP)
- **Group IV:** GSE (400 mg kg^{-1} IP)
- **Group V:** GSE: (600 mg kg^{-1} IP)

About 30 min later, 0.1 mL of formalin (2.5% solution in saline) was injected in the right hind paw of animals and 1-3 h later, the paw volumes were measured. An Ugo basile plethysmometer (model 7140, Millan Italy) was used to measure the paw volumes. The volume of paw recorded just before formalin injection was recorded as initial Volume (Vo) in each case. The mean percent rise in the paw volume was determined by following formula where Vt indicates volume at different intervals after formalin injection:

$$\text{Rise (\%)} = \frac{Vt - Vo}{Vo} \times 100$$

Statistical analysis: For statistical comparison, 3rd h of readings of percentage change in the paw volume were considered. The statistical analysis was carried out by one-way Analysis of Variances (ANOVA) ($p<0.05$) was taken to be statistically significant.

RESULTS

The effects of grape seed extract on formalin-induced rat paw edema are shown in Fig. 1 as percentage rise

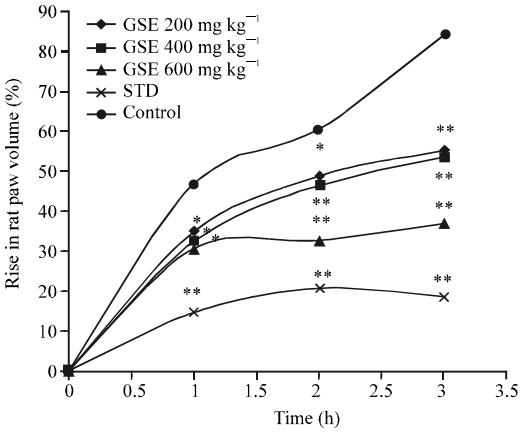

Fig. 1: Effect of grape seed extract in different doses on induced paw edema and comparison with positive and negative control groups (GSE = Grape Seed Extract, control = saline, STD = Standard group (indomethacin 5 mg kg^{-1}); *$p<0.05$; **$p<0.01$

Table 1: The percentage of rising in rats paw volumes at 3rd h

Treatment groups	Rise in paw volume (%)
GSE 200 mg kg^{-1}	55.02±5.6*
GSE 400 mg kg^{-1}	53.36±5.4*
GSE 600 mg kg^{-1}	36.98±4.5*
Indomethacin 5 mg kg^{-1}	18.53±2.3*
Saline (control)	83.97±6.5

n = 6 animals in each group. *Post test: $p<0.05$ as compare to control group; values are means±SEM

in paw volume >3 h following formalin injection. Figure 1 shows that all doses of grape seed extract exerted anti-inflammatory effect and this effect was dose dependent. Grape seed extract in dose of 600 mg kg^{-1} was more effective against induced paw inflammation. However, this effect was less potent than indomethacin (5 mg kg^{-1}) effect as positive control group.

Table 1 shows the average 3rd h percentage rise in paw volume in all treatment groups statistical test was applied to compare the treatment groups and control group. It was observed that the anti-inflammatory effects of grape seed extract in all administered doses were statistically significant as compare with saline group (Dunnett's test).

DISCUSSION

Inflammation is a non-specific symptom of many diseases and in some diseases such as autoimmune diseases inflammation is the most important feature of diseases and need to be treated with a therapeutic agent with minimum adverse effect. In this study, we investigated anti-inflammatory effect of grape seed extract in induced rat paw edema and the results showed that GSE has significant anti-inflammatory effect.

This study dose not involve elucidation of the mechanism of the anti-inflammatory effect of GSE. But with considering of inflammation mechanisme that the early phase of inflammation associated with release of substances such as histamine, prostaglandine and serotonin GSE may inhibit the releasing of these substances. Proanthocyanidins are the most abundant phenolic compounds in grape seeds and are high-molecular-weight polymers comprised of dimmers or trimers of (+)-catechin and (−)-epicatechin (Bagchi *et al*., 2000; Fine, 2000).

GSPE has various biological functions such as antibacterial, antiviral, anti-inflammatory, antiallergic and vasodilatoryactions (Chacon *et al*., 2009). It is reported that intraperitoneal injection of GSPE attenuated (Collagen-induced arthritis) CIA in mice and GSPE may be useful in the treatment of rheumatoid arthritis (Terra *et al*., 2009). Then, it is possible that GSPE is the most effective anti-inflammatory compound in grape seed extract and further studies is needed to verify it and compare the effect of GSPE and GSE in inflammation maybe elucidate the anti-inflammatory mechanism of GSE.

CONCLUSION

Grape seed extract recently has been formulated and used in different countries. Side effects of this product is seems to be very low. Therefore, we suggest the use of GSE as supplemental drug in patients with acute or chronic inflammatory diseases.

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