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Anti-inflammatory Effect of Grape Seed Proanthocyanidin Extract

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Abstract

The present study was carried out to evaluate the anti-inflammatory effect of grape seed proanthocyanidin extract. This study was performed by determined the thickness of paw skin (Carrageenan Test) and differential count of WBC (Basophil, Esinophil, Neutrophil, Monocyte and Lymphocyte) in blood serum samples in mice model. Twenty eight (28) male mice were divided into four equal groups and used for anti-inflammatory effects. First group (Control) received distilled water only, second group treated with 200 mg/kg of grape seed extract (GSE), third group treated with 200 mg/kg of grape seed proanthocyanidin extract (GSPE). Fourth group treated with indomethacin (0.36 mg/kg B.W). Fifty microliters of carrageenan (1%) was injected intradermally in the paw of mice after (30) minutes following the above treatments which administrated orally. The results showed that the animals that treated with carrageenan were suffered from increasing in the inflammation by significant increasing in skin thickness and also, significant differences in the differential count of WBC (Basophil, Esinophil, Neutrophil, Monocyte and Lymphocyte) in blood serum samples. While grape seed extract (GSE), grape seed proanthocyanidin extract (GSPE) showed decreasing in the thickness of skin and differences in the counts of Basophil, Esinophil, Neutrophil, Monocyte and Lymphocyte as compared with indomethacin. In this study the grape seed extract (GSE) have ability to reduce the inflammation that induced by carrageenan when compared with control group, the animals

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that treated with proanthocyanidin extract (GSPE) and the animals that treated with indomethacin.

Key words: Anti-inflammatory, Grape Seed, Proanthocyanidin

Introduction

Inflammation characterized by increased capillary permeability that is triggered by the activation of mast cells, followed by recruitment of neutrophils and macrophages [1]and[2]. Furthermore, selective inhibition of activation and/ or recruitment of inflammatory cells, pharmacological modulation of oxidative stress and expression of proinflammatory cytokines ameliorates hypersensitivity following injury [3].

Grape seed extract (GSE) is obtained from the waste product of grape juice, consists of various polyphenolic compounds such as catechin, epicatechin, gallic acid and abundant of flavonoids and polyphenols which consist of about 80%–90% of proanthocyanidins, and is known as grape seed proanthocyanidin extract (GSPE). Polyphenols are the main compounds present in GPSE which are responsible for an anti-inflammatory action. GSPE has shown to inhibit the release of various excitatory cytokines [substance P, calcitonin gene related peptide (CGRP), cytokines] which exhibits reduction in the expression of various soluble adhesion molecules. GPSE is also responsible for increasing the levels of endogenous anti-oxidants, i.e. superoxide dismutase (SOD), catalase and reduced glutathione (GSH). In addition to their free radical scavenging and anti-oxidant activity, proanthocyanidins have been reported to have antibacterial, antiviral, anticarcinogenic, anti-allergic and vasodilatory actions [4]. Hence, this study aimed to evaluating the anti-inflammatory effect of grape seed proanthocyanidin extract (GSPE) and the differences between the crude (GSE) and proanthocyanidin extract of grape seeds in the relief of inflammation.

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Materials and Methods

Plant Materials

Red grape seeds which were obtained from local market were handily separate from grape skin and stem (waste), were collected in October 2020 from the alkut region.The classification of the plant was done in the university of Baghdad /collage of science

Extraction and Isolation

Red grape seeds which were obtained from local market will handily separate from grape skin and stem (waste), then were washed with tap water and then left to dry in open air away from direct sunlight .They were crashed in a coffee grinder for 2 min, but during this time the grinding will halted for 15 sec at periodic intervals to prevent heating of the sample. The samples were wrap and stored at -18 c until the extraction was performed. The extract of grape seeds was obtained by ethanol 70% with ratio 8:1 of liquid to solid at 50c for 3 hours by magnetic stirrer [5]. Then put in rotary evaporator to dry extract then stored in the freezer (-20).

Animals:

28 male albino mice, three months old and weighed about 29 ± 3g were obtained from Samarra Drugs Industries.

The mice were randomly divided into five main groups each group have 28 animals and housed in stainless steel wire mesh cages on a bedding of wood shavings. Ambient temperature was controlled at (25 ± 3 C) with relative humidity of 50% ± 15% and a light/ dark cycle of 12 hrs./12 hrs. Food and water were provided at all times except before drugs administration (1/2 hrs. before administration).

Preparation of the Stock Solution, Concentrations and Doses of Grape Seed Extract (GSE):

The dilutions of the (GSE) were prepared by weighing the amount of (200mg) of this extract and

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completed to (10) ml with distilled water, the final concentration was recorded and the final dose calculated was 0.1 ml /10 gm of rat body weight which was equal to the dose (200 mg/kg) of (GSE).

Preparation of the Stock Solution, Concentrations and Doses of grape seed proanthocyanidin extract (GSPE): The dilutions of the (GSPE) were prepared by weighing (200mg) of (GSPE) (company) and complete to (10) ml with distilled water, the final concentrations were recorded and the final dose calculated was ml /gm of rat body weight which was equal to the dose (200mg/kg) of (GSPE).

Preparation of the Stock Solution, Concentrations and Doses of Indomethacin : The dilutions of the indomethacin were prepared by weighing (3.6 mg) of indomethacin (company) and complete to (100) ml with distilled water, the final concentration was recorded and the final dose calculated was ml /gm of rat body weight which was equal to the dose (0.36mg/kg) of indomethacin.

Experimental Design:

28 male mice were divided into 4 equal groups and used for anti-inflammatory effect.

Anti-inflammatory effect of grape seed proanthocyanidin extract:

1-Determination of thickness of skin (Carrageenan Test) .

Anti-inflammatory effect was tested in 28male mice by carrageenan test as follows:

Group (1)	Group (2)	Group (3)	Group (4)
Treated orally with distilled water (control)	Treated orally with (GSE)	Treated orally with 200 mg/kg (GSPE)	Indomethacine 0.36 mg/kg B.W.orally [6]

N=7

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Fifty microliters of carrageenan (1%) was injected intradermally in the paw of mice after (30) minutes following the above treatments .

The experimental models of local inflammation due to carrageenan (a vegetable product that causes local inflammation) [7] was developed in the hind paw in mice. In this study a model was developed for a local inflammation induced by carrageenan in mice hind paw , causing dermatitis as it was able to demonstrate by measuring thickness of skin by micrometer **,** and demonstrate by a differential count of WBC as well as by studying the effect of different substances in this model.

2-Differential count of WBC (Basophil, Esinophil, Neutrophil, Monocyte and Lymphocyte).

Blood Serum Samples

Blood samples were collected after the treating from mice heart directly by (insulin syringe) in a dry, clean and sterile centrifuge tubes (EDTA tubes), and then left few in refrigerator till performing the differential white blood cells analysis [8].

Differential count of WBCs:

The anti-inflammatory effect of (GSE), (GSPE) and Indomethacin demonstrated by differential count of inflammatory cell mainly WBCs. The method was mentioned by [9] , that include one drop of blood was taken in two hour from injection of carrageenan was dripped on the slide then this drop was spread with one stroke and after getting dried , a slide was dyed with Leishman's stain and left for 10 min. , then washed with tap water . The slide was examined under the microscope (power of adjustment was 100). It was examined in zigzag like line up and down until 100 cells were counted (every type was identified to calculate the percentage of cells).

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Statistical Analysis

The Statistical Analysis System- SAS (2012) program[10] was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means in this study.

Ethics

The handling of animals and the experimental protocol are performed for being sure that animals do not suffer at any stage of the experiments.

Results & Discussion

Extraction (by ethanol 70%) grape seeds

The extraction ratio was 25.2 % from Grape seeds, the color of the extract was dark red and the texture was semi gelatinous and sticky (figure 1).

Figure (1): Appearance of ethanolic extract of Grape Seeds

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Anti-inflammatory effect of grape seed proanthocyanidin extract:

1 : Determination of Thickness of skin (Carrageenan Test) .

This test revealed significant increase (P≤0.05) in the time needed by all animals of treatment groups GSPE extract , natural extract and Indomethacin to increase thickness of skin throughout the period of study (after 5 minutes, after 10 minutes, after 15 minutes and after 30 minutes) in comparison with the time needed by animals of the control one. Within groups the results showed skin was start thickness after 5 minutes in GSPE extract , natural extract and Indomethacin groups the same pattern of significant increases (P≤0.05) while skin of control group start thickness after 30 minutes , after 15 minutes the natural extract (2.52 ±0.10) and Indomethacin (2.50 ±0.16) showed significant deference in compered with control group (2.93 ±0.13) but there was no deference between theme ,and after 30 minutes the treatment groups(2.45 ±0.16 , 2.22 ±0.06 and 2.08 ±0.10) show significant deference at (P≤0.05) than control group (3.30 ±016) with no deference between treatment groups as showed in table (1).

Table (1): Anti-inflammatory effect (Carrageenan Test) to determine the effect of natural extract along with GSPE extract in comparison with indomethacin

Groups of mice	Thickness of skin(mm)					LSD value
	Before treatment	After 5 min.	After 10 min.	After 15 min.	After 30 min.	
Control Group	1.21 ±0.03 B a	2.34 ±0.16 B a	2.70 ±0.08 AB a	2.93 ±0.13 AB a	3.30 ±016 A a	0.511 *
Treated orally with (GSPE)	1.21 ±0.04 B a	2.33 ±0.30 A a	2.72 ±0.23 A a	2.66 ±0.13 A ab	2.45 ±0.16 A b	0.548 *
Treated orally with (GSE)	1.20 ±0.07 B a	2.63 ±0.17 A a	2.59 ±0.10 A a	2.52 ±0.10 A b	2.22 ±0.06 A b	0.602 *
Indomethacin	1.20 ±0.02 B a	2.20 ±0.18 A a	2.52 ±0.21 A a	2.50 ±0.16 A b	2.08 ±0.10 A b	0.541 *
LSD value	0.159 NS	0.653 NS	0.505 NS	0.398 *	0.397 *	---

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Means with different small letters in the same column and big letters in the same row are significantly different. * (P≤0.05). N=7

2-Differential count of WBC (Basophil, Esinophil, Neutrophil, Monocyte and Lymphocyte).

The result of differential of WBC of treatment group in compered with control group as fallowing : the Lymphocytes record no significant deference between treatment group while the Natural extract group was deference significantly at (P≤0.05) (27.74 ±0.98) in compered with control group (24.29 ±1.29);in Monocytes the GSPE extract record the highest vale between treatment group(4.54 ±0.09) but there is no significant deference in compered with control group (5.07 ±0.24), the Natural extract (2.84 ±0.32) and Indomethacin (3.27 ±0.39) ware deferent than control group (5.07 ±0.24) at significantly (P≤0.05) but no deference between them; the Neutrophils was record no significant deference between treatment and control group ; in the Eosinophil the highest value was record in control group (0.802 ±0.04) and the lowest value in Indomethacin group (0.361 ±0.04) while the GSPE extract(0.564 ±0.06) and Natural extract(0.501 ±0.07) there was no significant deference between them but in compered with control group (0.802 ±0.04) there was significant deference at (P≤0.05); the Basophils was not appeared in all groups as show in (table:2).

Table (2): differential of WBC /DLC to determine the effect of natural extract along with GSPE extract in comparison with indomethacin

Groups	Lymphocytes %	Monocytes %	Neutrophils %	Eosinophil %	Basophils %
Control	24.29 ±1.29 b	5.07 ±0.24 a	69.83 ±1.25 a	0.802 ±0.04 a	0.00 ±0.00 a
Treated orally with (GSPE)	25.96 ±0.61 ab	4.54 ±0.09 a	68.92 ±0.56 a	0.564 ±0.06 b	0.00 ±0.00 ± a
Treated orally with (GSE)	27.74 ±0.98 a	2.84 ±0.32 b	68.92 ±1.01 a	0.501 ±0.07 b	0.00 ±0.00 ± a

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Indomethacin	26.95 ±0.81 ab	3.27 ±0.39 b	69.41 ±1.05 a	0.361 ±0.04 c	0.00 ±0.00 ± a
LSD value	2.806 *	0.818 *	2.940 NS	0.174 *	0.00 NS
Means having with the different letters in same column differed significantly. * (P≤0.05). N=7					

The results of this study showed that carrageenan resulted in redness; swelling, hotness and painful edematous paw tissue with a significant increase in edema till reach the maximum after 30 minute post-injection and the same signs was recorded in [11] which were attributed this effects to the acute inflammatory response induced by carrageenan which characterized by the exudation of tissue fluids and plasma resulting in edema formation and concurrent accumulation of leukocytes mainly neutrophils .The anti-inflammatory activity of indomethacin was evaluated by using carrageenan-induced paw edema test showed that the edema start reduction after 30 min basted on paw-thickness due to its activity as anti-inflammatory effect by working on prevent stimulation and action of polymorphonuclear leukocytes and may inhibit expression of cell adhesion molecules [12], cell adhesion molecule plays a role in inflammation and cancer[13]. Similar study by [14] which recorded reduction in the inflammation signs after one hour. In our study the grape extract showed decrease in the edema and inflammation in compare with control group and this agree with [15] who explained this effect by the role of proanthocyanidin that help in the promotion of collagen formation and elastin and preservation of skin moisture. Furthermore, proanthocyanidins induce vascular endothelial growth factor, reduces edema and encourages blood flow. We can attributed the anti-inflammatory effect of the grape seeds extracts to the ability of the in the decreasing the counts of inflammatory cells, which responsible for producing the inflammatory cytokines [16].

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