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Effects of dietary phytocompounds in a guinea pig model of paw edema

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Abstract: Background**:** Grape seed powder (GSP) and Green tea powder (GTP) are the two popular natural sources that have the potential to attenuate inflammation. The development of natural therapeutic agents for inflammation associated disorders will reduce conventional chemicals. Objective: The present study was designed to determine if a diet enriched with GSP and GTP would alter the carrageenan-induced paw edema in the guinea pig model. Method: Diets containing varying amounts of GSP (250mg-500 mg/kg BW) and GTP (250 mg-500 mg/kg BW) were prepared. The carrageenan-induced Dunkin Hartley guinea pig paw edema model was used to study the effect of GSP and GTP on acute inflammation. Animal groups fed a standard diet supplemented with low (250 mg/kg/day× 14 days) and high dose (500 mg/kg/day× 14 days) of GSP and GTP for two weeks. On day 15, edema was induced; paw volume and percentage inhibition rate of edema was calculated. Afterward, Prostaglandin E2 was estimated in the serum of control and inflamed guinea pig. Results: In multiple comparisons between GSP and GTP, at dose of 250mg/kg both gives insignificance ($p>0.05$) difference in edema paw volume. Although, GSP and GTP showed a significant difference ($p=0.01$) in edema paw volume at a higher dose of 500mg/kg. GSP produced a significantly greater decrease in PEG2 level than GTP in a dose-dependent manner. At dose 250 mg/kg GSP and GTP did not show a significant difference in PGE2 level ($p>0.05$) while at dose 500 mg/kg both phytocompounds showed significant difference ($p=0.01$). Conclusion: The results indicate that a diet enriched with phytocompounds showed increased percentage inhibition of paw edema in a dose-dependent manner on carrageenan-induced paw edema in guinea pig. GSP and GTP showed dose-dependent inhibitory activity on PGE2 production in the serum of inflamed guinea pigs.

Keywords*:* Edema, dose dependent, phytocompounds, carrageenan

INTRODUCTION

Herbal antioxidants as mono-therapy relieve many inflammatory diseases such as arthritis, muscle pain, sore throat, toothache, and fever. Inflammation is the response of a tissue and its microcirculation to pathogenic injury. It is characterized by the generation of inflammatory mediators and movement of fluid and leukocytes from the blood into extravascular tissues which give rise to the signs of inflammation [1]. Medicinal plants that have pharmacological evidences

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supporting their uses in folk medicines as antioxidant and anti-inflammatory agents may serve as valuable sources of natural medical therapies for inflammatory diseases [2].

Grape seed powder (GSP) is a natural mixture from the seeds of *Vitis vinifera* containing high polyphenol level. GPS exhibiting numerous health-promoting effects and various biological functions such as antioxidant, anti-inflammatory, anti-allergic and vasodilatory properties. GSP is also used as a nutritional supplement and also has Generally Recognized as Safe (GRAS) status approved by the Food and Drug Administration [3,4]. The safety of GSP had already been evaluated by Charradi et al by administering high and repeated dosing to healthy rats in a two-month-long sub-chronic experiment. Data shows high dosage GSP (4g/kg BW) was safe and well-tolerated and should be considered as a multi-targeted and multi-faceted mixture of bioactive compounds [3]. On the other hand, tea is a popular drink among people as a healthy consumption. Green tea (*Camellia sinensis*) catechins have GRAS status and demonstrated versatility in providing health benefits [4,5]. Overly, data reported that green tea leaves contain considerable amounts of protein, fiber, minerals, and bioactive compounds especially tannins and saponins [6]. Previous findings have indicated green tea leaves possessed a marked anti-inflammatory effect against the in vitro denaturation of protein and suppress the gene or protein expression of inflammatory cytokines and inflammation-related enzymes [7]. Prostaglandins play a key role in the generation of the inflammatory response. Prostaglandin E2 (PGE2) is one of the most abundant prostaglandin produced in the body, is most widely characterized in animal species, and exhibits versatile biological activities. In inflammation, PGE2 is of particular interest because it is involved in all processes leading to the classic signs of inflammation: redness, swelling, and pain. Redness and edema result from increased blood flow into the inflamed tissue through PGE2-mediated augmentation of arterial dilatation and increased microvascular permeability [8].

In the present study, it was found worthwhile to determine if a diet enriched with GSP and GTP would alter the carrageenan-induced paw edema in the guinea pig model.

MATERIALS AND METHODS

Material: Non-gelling λ-Carrageenan powder was procured from Sigma Aldrich India. Liquid Scintillation Counter Pro-Pack (Pro-Pack materials India Pvt Ltd). Unlabelled PGE2 and anti-PGE2 were obtained from Sigma Chemical India. Dextran-coated charcoal (0.4% dextran, 2% charcoal) was used to separate the free and bound ligands. Commercial liquid scintillation cocktail (EcoLite(+) Scintillation Cocktail) purchased from MP Biomedicals (India Pvt Ltd).

GSP and GTP containing diets: Grapes seeds were procured from a grape cultivar of *Vitis vinifera* from northern Andhra Pradesh. Air-dried seeds were grounded with an electric grinder until a fine powder was obtained. Young and green leaves of tea *Camellia sinensis* were collected from the Kangra district of Himachal Pradesh India. Leaves were properly washed to remove the dust particles, shade dried, cut into small pieces, and pulverized into fine powder using a mechanical grinder powdered with the help of a grinder. Grapes seeds powder (GSP) and green tea leaves powder (GTP) were passed through #85 and stored [9]. Dosing of GSP and GTP was selected based on its known traditional usage [3,10,11]. Diets containing varying amounts of GSP (250mg-500 mg/kg BW) and GTP (250 mg-500 mg/kg BW) were prepared. GSP was mixed to an already ground standard rodent chow and an equal volume of sterile distilled water. Then diets in pellet form were reconstituted using an electric mixer and dried in an oven at 40°C until weight stabilization indicating complete dehydration 3. The same procedure was followed for GTP containing diet.

Carrageenan-induced paw edema in guinea pig: The carrageenan-induced Dunkin Hartley guinea pig paw edema model was used to study the effect of GSP and GTP on acute inflammation. Albino guinea pig of either sex were used in this study in conformity with the institutional animal ethical committee (1044/PO/Re/S/07/CPCSEA, PHARM/01/IAEC/2017) and in agreement with the CPCSEA guidelines India.Dunkin Hartley guinea pigs weighed 535±10.54 g at the start

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of the study and were housed in a sanitized aluminum cage with sawdust bedding. The ambiance of the experimental animal room was 20±4°C temperature and 50±15% RH, a 12h light/dark cycle. After acclimatization of one-week animals were divided into 5 groups of 6 animals each. One group kept as control and fed a standard diet, rest four groups fed a standard diet supplemented with low (250 mg/kg/day× 14 days) and high dose (500 mg/kg/day× 14 days) of GSP and GTP for two weeks with an unrestricted supply of drinking water [12]. On day 15, edema was induced by injecting 20µL of carrageenan solution (1.0%, w/v) into sub plantar area of the right hind paw of all thirty animals [13]. The preparation of the carrageenan solution was based on the method described by Fehrenbacher et al [14]. Animals were weighed and marks were made on the right hind paw just behind the tibia-tarsal junction on each animal. Measurement of paw volume was carried out with caliper rule (Digimatic, Mitutoyo 500, Japan) after 4h following carrageenan injection. The extent of the edema was measured by the difference in size between the two hind paws. Thus, every time the paw was dipped in the plethysmograph (mercury displacement method) up to the fixed mark to ensure constant paw volume. Subsequently percentage inhibition rate of edema was calculated by using formula [15]. % inhibition=100(1-Vt/Vc), where Vc represents edema volume in control and Vt the edema volume in the group treated with GSP and GTP.

Prostaglandin E2 estimation in serum of control and inflamed guinea pig: After 4 hours of carrageenan injection, blood samples of all the animal groups were also collected in eppindorf tube. The serum was separated by centrifugation without delay at 1500×g for 15 minutes at 4°C and stored frozen at -20°C until assayed for PGE2 using radioimmunoassay. Radioimmunoassay procedures were carried out in triplicate for each compound.Preparation of StandardsA series of concentrations of PGE2 standards were prepared, ranging from 2.45–240 picogram (pg)/0.1 ml. 100 µL of PGE2 standard solution was added to 100 µL of anti-PGE2. The mixture was incubated at 4°C for 24 h. After incubation, the mixtures were added with 200 µL of dextran charcoal and were incubated again for 10 min. After centrifugation at 3000×g for 15 min at 4°C, 2 ml of liquid scintillation cocktail was added to 200 µl of supernatant. The radioactivity was measured by a liquid scintillation counter [16].The reaction mixtures consisted of 100 µl of serum, 100 µl of anti-PGE_2 were incubated at 4°C for 24 h. After incubation, the mixtures were added with 200 µl of dextran charcoal and were incubated again for 15 min. After centrifugation at 3000 × g for 20 min at 4°C, 3 ml of liquid scintillation cocktail was added to 300 µl of supernatant. The radioactivity was measured by a liquid scintillation counter [16,17].The readings obtained for each set of triplicates were averaged. The net counts for all standards and samples were calculated by subtracting the value of the antibody binding to the antigen in the sample with nonspecific binding.

Statistical analysis: The data were presented as mean± S.E.M. and analyzed statistically by one-way analysis of variance followed by post-hoc Tukey's multiple comparison test to evaluate the significant differences ($P<0.05$) between the mean values.

RESULTS AND DISCUSSION

GSP and GTP are the two popular natural sources that have the potential to attenuate inflammation. In the carrageenan-induced edema test, the paw volume and percentage inhibition by GSP and GTP are shown in table 1. Sub plantar injection of carrageenan into the hind paw induced a progressive edema gaining its maximum at 4 hours. The degree of swelling depends on the carrageenan preparation. A maximum increase of paw thickness, 6.22±0.36 mm, was obtained 4h following carrageenan injection in control pig. GSP significantly attenuated paw volume at dose-dependent manner as compared to the control pig ($P<0.05$). GTP also showed a significant effect ($p<0.05$) against carrageen-induced paw edema in the guinea pig model. In multiple comparisons between GSP and GTP, at a dose of 250mg/kg, both give insignificance ($p>0.05$) difference in edema paw volume. Although, GSP and GTP showed a significant difference ($p=0.01$) in edema paw volume at a higher dose of 500mg/kg. From these results, it is believed

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that GSP has more potential anti-inflammatory activity at dose dependent manner against carrageen induced edema in guinea pig model.

Table 1. Effect of GSP and GTP on carrageenan-induced paw edema in guinea pig

Group	Dose (mg/kg)	Paw Volume (mm)	Inhibition rate (%)
Standard diet (Control inflamed)	-	6.22±0.36	0
GSP enriched diet	250	3.13±0.06^{ns}	49.67^{ns}
	500	1.81±0.56**	70.90**
GTP enriched diet	250	3.60±0.85^{ns}	42.12^{ns}
	500	2.72±0.46**	56.27**
comparison between different dosages of GSP&GTP within same column ns $p>0.05$, **$p\leq0.01$			

Serum levels of PEG2 of different experimental groups are present in Table 2. The prostaglandin levels in control inflamed guinea pigs were significantly higher ($p=0.001$) than GSP and GTP diet animal groups. Previously Turull et. al have also been shown increased PEG2 production in inflamed paws of adjuvant arthritic rates [18].Serum PEG2 level in the control (healthy) guinea pig was 270.22±32.45 pg/ml. GSP (250-500 mg/kg) attenuated the elevated PGE2 level by 406.12±12.90-286.04±24.24 pg/ml. Results show GSP produced a significantly greater decrease in PEG2 level than GTP in a dose-dependent manner. At dose 250 mg/kg GSP and GTP did not show a significant difference in PGE2 level ($p>0.05$) while at dose 500 mg/kg both phytocompounds showed significant difference ($p=0.01$). In our data inhibition of PEG2 level in serum of GSP and GTP diet animals is proportional to the inhibitory effect of edema volume. Both the phytocompounds GSP and GTP studied here did not reduce the PEG2 level in experimental animals below that of healthy control.

Table 2. The mean (±S.E.M) serum PEG2 level in control and experimental animal

Group	Dose (mg/kg)	Serum PGE2 level (pg/ml)
Standard diet (Control healthy)	-	270.22±32.45
Standard diet (Control inflamed)	-	617.03±12.78
GSP enriched diet	250	406.12±12.90
	500	286.04±24.24
GTP enriched diet	250	437.12±32.67
	500	341.31±33.87

Fig 1 shows the percentage change in the PGE2 level in experimental groups compared with the healthy control group.Previously Sajali et al stated that flavonoids can regulate arachidonic acid metabolism via inhibition of cyclooxygenase-2(COX-2) activity from different sources [16]. Tea leaves are a good source of flavonoids, indicating that is might directly inhibit COX-2 enzymatic activity. Therefore, the compounds which inhibit the production of PGE2 may reduce the inflammation responses. Grape seeds contain polyphenol-rich phytocompounds such as proanthocyanidins (oligomeric flavonoids), flavonoids (catechin, epicatechin, and quercetin), and anthocyanins. Some researchers have also been reported that procyanidins from GSP significantly inhibited expression of COX-2 and PGE2 in mouse skin tumor and rat intestinal mucosal injury [19,21].

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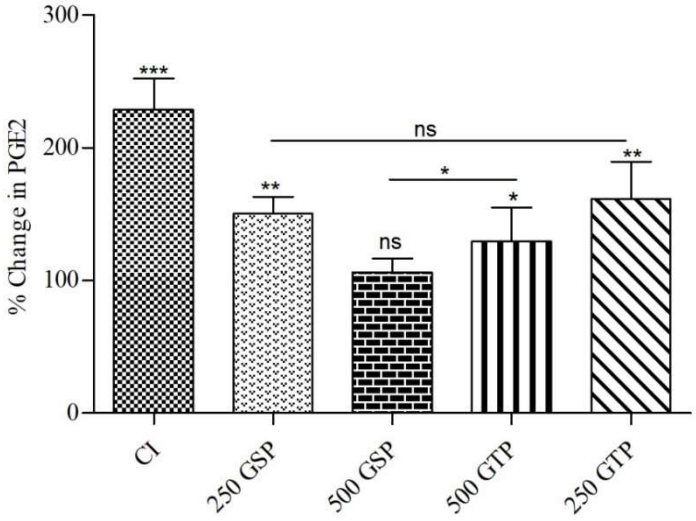

Fig. 1 Percentage change in PGE2 level in experimental groups compared with healthy control group. Where CI is control inflammed, values plotted are mean±SEM (n=6). ns $p>0.05$,***<0.001, **<0.01,*<0.05

CONCLUSION

The results indicate that a diet enriched with phytocompounds showed increased percentage inhibition of paw edema in a dose-dependent manner on carrageenan-induced paw edema in guinea pig. GSP and GTP showed dose-dependent inhibitory activity on PGE2 production in the serum of inflamed guinea pigs. Both appear as a good candidate for large sample size experiments for the development of natural therapeutic agents for inflammation-associated disorders which reduces conventional chemicals.

CONFLICTS OF INTERESTS

The authors declare no conflict of interest.

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