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Full Length Research Paper

Beneficial effects of *Solanum melongena* (Solanaceae) peduncles extracts, in periodontal diseases

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The antioxidant activity of aqueous extracts from S*olanum melongena* L. fruit (egg plant) and from its peduncles were investigated *in vitro* using electrolysis as a free radical generating system. The peduncles extracts have been then given to twenty volunteer patients with periodontal diseases as a mouthwash solution for 3 months duration, against a placebo, in a double blind fashion. Saliva was collected before and after the mouthwash using a Salivette tube. The antioxidant activity of saliva was determined *in vitro* using the electrolysis system. In addition, total antioxidant activity as well as glutathione levels of the saliva were evaluated before and after the mouthwash in comparison with 10 healthy individuals. The results indicated that water extracts from the peduncles of *S. melongena* possessed a higher capacity to scavenge free radicals than the fruit itself. They as well increased the total antioxidant activity and glutathione levels of saliva in patients with periodontal diseases, while it was not effective with the placebo. The extracts were also clinically effective, they ameliorated significantly pocket depth, and the bleeding index. We concluded that water extracts from peduncles of *S. melongena* used frequently as mouth wash may have beneficial effect against periodontal diseases.

Key words: Periodontal diseases, *Solanum melongena* extracts, free radicals, antioxidant activity.

INTRODUCTION

Oral cavity is the first to be exposed to bacteria that lodge between the teeth and the gum and attract the polymorphonuclear cells (PMN) from blood to gingival fluid. The activated PMN released oxygen free radicals (OFR) in order to digest the bacteria after phagocytosis. In certain circumstances when PMN are highly activated or being destroyed by bacteria, the OFR are generated by PMN but exudates outside the cell. Hence, OFR can attack the healthy cells of the gum leading to periodontal diseases. Nevertheless, saliva protects the oral cavity against the toxicity of OFR by a process, known as antioxidant activity of the saliva, whose mechanism is still not yet elucidated. If there is an imbalance between OFR production and the availability of antioxidants in the saliva, this phenomenon is closely related to the etiology of periodontal diseases (Edgar, 1992; Moore et al., 1994; Sculley and Langley-Evans, 2002).In a former study (Diab-Ladki et al., 2003) we have demonstrated that stimulated saliva from healthy individuals is significantly more effective (40 to 50%) than that from patients with periodontal diseases in scavenging a wide variety of free radicals generated *in vitro*. Moreover, the total antioxidant activity of saliva was significantly decreased in those patients. We concluded that periodontal diseases are associated with an imbalance between oxidants and antioxidants in favor of the first due to both an increase in free radical production and a defect in the total antioxidant activity of the saliva.

The present study is devoted to test *in vitro* the antioxidant activity of different extracts from S*olanum melongena* L. (eggplant) fruit with their peduncles rich in flavonoids, and to determine the ability of such extracts to ameliorate the antioxidant activity of saliva in a group of

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patients with periodontal diseases. Despite the absence of scientific evidence, the peduncle of eggplant are used traditionally in our region for the treatment of gum inflammation.

Eggplant is used mainly as a food crop, but it does also have various medicinal uses that make it a valuable addition to the diet. In particular the fruit helps to lower blood cholesterol levels (Guimarães et al., 2000). The fruit has antihaemorrhoidal and hypotensive effect. It is also used as an antidote to poisonous mushrooms. It is bruised with vinegar and used as a poultice for cracked nipples, burns, abscesses, cold sores. The leaves of the plant are also used for similar conditions, however they are toxic and should only be used externally. The ashes of the peduncle are used in the treatment of intestinal hemorrhages, piles and toothache. A decoction of the root is astringent (Sudheesh et al., 1999; Noda et al., 2000; Mutalik et al., 2003; Mans et al., 2004).

MATERIALS AND METHODS

Preparation of plant aqueous extract

Eggplants were furnished by a farmer from Bekaa region (Lebanon). On each occasion, 1 kg of the fresh fruit without peduncles or 1 kg of peduncles alone were washed with distilled water, cut into smaller pieces and ground in a commercial blender. The milled was soaked in hot distilled water and extracted twice, each time with 2.5 L of hot distilled water (at 90 to 100°C) for 12 h. The combined extracts were concentrated to dryness under reduced pressure in a rotary evaporator at 70 ± 1°C. The resulting crude aqueous extract was freeze dried, finally giving 98 g (9.8% yield) of a dark brown, powdery, aqueous extract residue. Without any further purification, aliquot portions of the residue were weighed and dissolved in distilled water (at room temperature) for daily experimental use (Pham et al., 1986), or as a mouth rinse.

Total phenolic content determination

Total phenolic content (TPC) was measured by the colorimetric method with Folin-Ciocalteu reagent (Slinkard and Singelton, 1997) and gallic acid was used as a standard. Total phenols were expressed as mg gallic acid equivalent per gram dry weight (mg GAE).

Participants and saliva collection

Stimulated saliva samples were collected from twenty patients with chronic periodontal diseases that require dental treatment (oral hygiene, gingival recession and more than half of attachment loss).

All participants of both gender were volunteers, aged between 30 and 45 years, and were in good overall health, not taking any medication interfering with saliva secretion. Ten patients were asked to use a mouth wash extracts from *S. melongena* 3 times a day for 3 months and 10 others a placebo in a double blind manner. Saliva samples were collected thereafter. As a control group, samples were also collected from 10 individuals with apparently healthy gums. Saliva samples were collected in the early morning (after tooth brushing and before breakfast). Salivation was induced by rolling a natural gum around the mouth for 5 min and saliva was collected in Salivette disposable tubes. Salivary volumes were measured and then saliva samples were stored at -80°C until assay (Diab-Ladki et al., 2003).

Clinical examination

Clinical data were collected at a pre-treatment baseline appointment and at a three month post-treatment appointments. One examiner was responsible for all the clinical measurements and saliva sampling at baseline as well as the re-examination. Clinical data were recorded from all teeth except third molars and severely malpositioned teeth. (Lenox and Kopczyk, 1973; Lopez et al., 2000). The following parameters were considered:

1. Pocket depth (PD) in mm was recorded from four surfaces of each tooth distobuccal, mid-buccal, mesiobuccal, and mid-lingual using a periodontal probe (Hu Friedy, Chicago, IL, USA).
2. The bleeding index (BI) was calculated as the percent of bleeding points recorded 30 seconds after probing.

Oral hygiene instruction cleaning method was performed for all patients. On the same day patients received randomly the extract or the identical placebo. An assistant used to call all participants every day to remind them to respect the protocol. Three months later, the patients were called for a complete mouth examination and saliva sampling.

Free radicals generating system

The antioxidant capacity of *S. melongena* extracts and that of saliva was evaluated *in vitro* using electrolysis (two platinum wire electrodes placed in 20 ml Tyrode buffer solution to generate FR and their by-products). A constant 10 mA direct current generated by a stimulator was applied for 5 min in the absence or presence of 0.1, 0.2 or 0.4 ml of plant extracts or saliva of each sample. The amount of OFR and their by-products was determined by a colorimetric method using N, N - diethyl - P - phenylendiamine (DPD). A volume of 1 ml of electrolysis sample was added to 2 ml DPD (25 mg/ml) dissolved in the buffer at the end of electrolysis. The electrolysis-induced oxidant species reacted instantly with DPD reagent to produce a red color measurable at 515 nm (Lecour et al., 1998).

Biochemical assays of saliva

Total antioxidant activity of saliva (TAA)

The total antioxidant activity of saliva before and after mouth wash was estimated by the ABTS assay (Randox Lab) which involved the interaction of ferrylmyoglobin radical, produced by the activation of metmyoglobin with phenothiazine. ABTS [2,2'-azinobis-(3-ethylbenzo-thialozine-6-sulphonic acid] produced the ABTS radical cation. This blue chromogene exhibited characteristic absorption at 660 nm. In the presence of antioxidants, the amounts of ABTS radical cation produced were decreased. Thus the absorption was inhibited dependently on the total antioxidant capacity. The assay was standardized using the vitamin E analogue Trolox (Diab-Ladki et al., 2003, Kondakova et al., 1999).

Determination of glutathione in saliva

We used a kit colorimetric method employing a kinetic

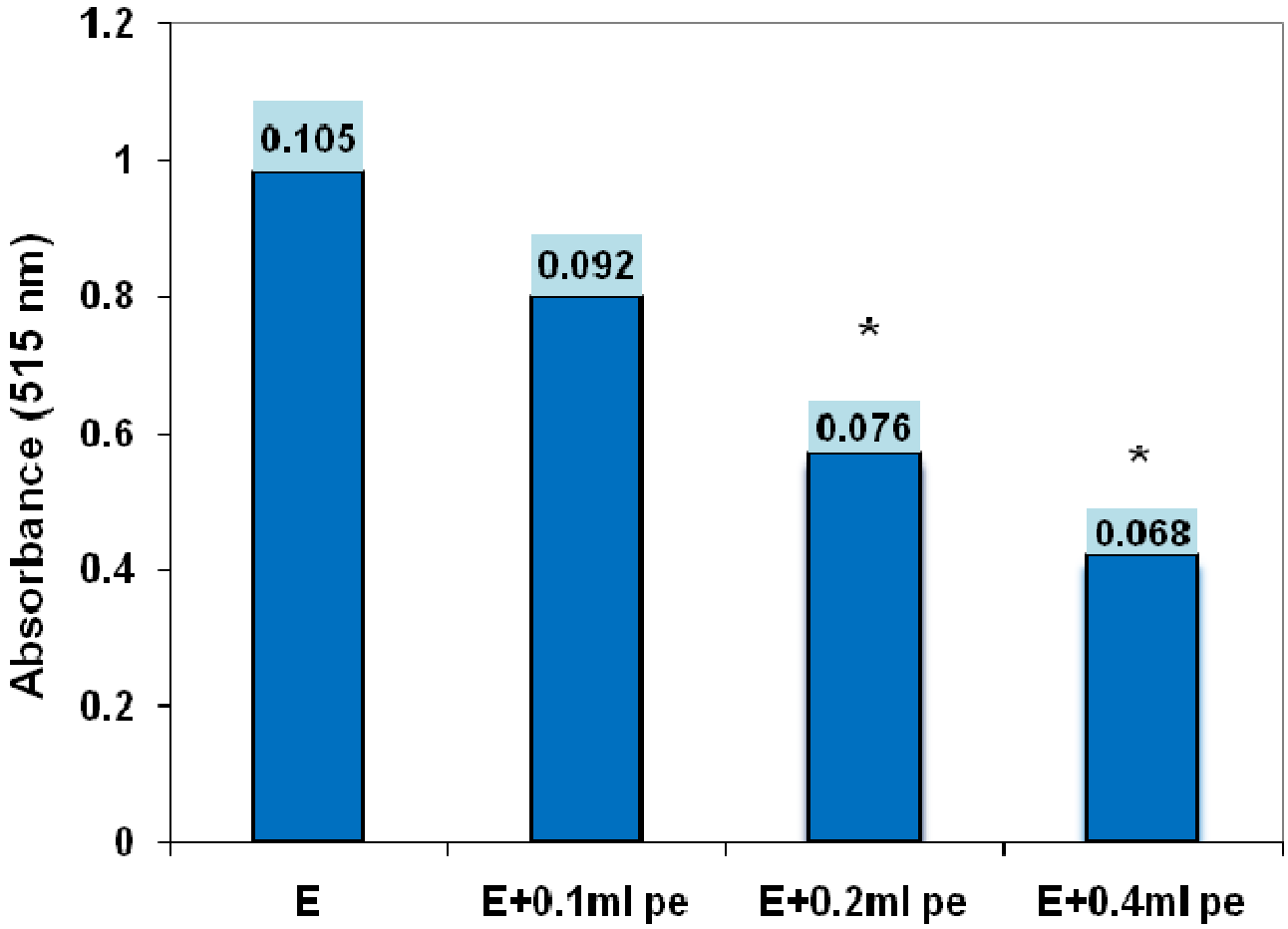

Figure 1. Antioxidant activity of different concentration from *Solanum melongena* fruit extracts (fe) without peduncles (pe) against free radicals generated *in vitro* by electrolysis (E) of a physiological buffer solution. 1 ml of the aqueous solution (fe) contain 98 mg of powder extract Values represent means ± SD of 6 experiments after 5 minutes electrolysis. * $p<0.05$ versus E.

enzymatic recycling assay, based on the oxidation of GSH by 5,5'-dithiobis-(2-nitrobenzoic acid), [DTNB] to measure the total glutathione (tGSH) content of biological samples. Glutathione standards or treated samples were added to the microtiter plate wells, followed by DTNB and glutathione reductase. Addition of NADPH2 to the wells initiated the progressive reduction of DTNB by GSH, causing a color increase that was monitored at 405 nm. The rate of color change, typically followed over a 4 min time period, is proportional to the tGSH concentration. Consequently, the concentration of tGSH in the unknown samples may be determined by reference to the standard curve. GSH reacted with DTNB to produce both a colored ion, which absorbed light at 405 nm, and a mixed disulphide. The disulphide reacted with further quantities of GSH present to liberate another ion and GSSG. GSSG is reduced enzymatically to GSH which then re-entered the cycle. Since GSSG represented only a small percentage of total acid-solution free glutathione, the resulting values for tGSH (which encompasses both GSH and GSSG) are expressed in units of GSH equivalents (Pastore et al., 2003).

Statistical analysis

A Student's *t*-test was used to evaluate statistical differences between the groups. The level of significance was set at 5%.

RESULTS

In Figures 1 and 2 are represented the antioxidant activity of extracts from *S. melongena* fruit without peduncles and from peduncles alone at the fifth minute electrolysis of a solution buffer. The absorbance of the buffer solution (control) was 0.98 ± 0.105. The addition of extracts decreased the absorbance in a dose dependent manner. For instance, 0.4 ml of peduncle extracts (absorbance: 0.42 ± 0.068) were more effective in scavenging the radicals produced by electrolysis comparatively to 0.4 ml of extracts without peduncles (absorbance: 0.59 ± 0.073). It is important to mention that 1 ml of aqueous solution contain 98 mg of powder extract. In addition, the extract from fruit without peduncles has a lower TPC value of 28.7 mg GAE/g dry weight, whereas 47.5 mg GAE/g dry weight was detected in the peduncles extracts. They were used thereafter as a mouth wash solution. In Figure 3, the absorbance decreased in the electrolysis

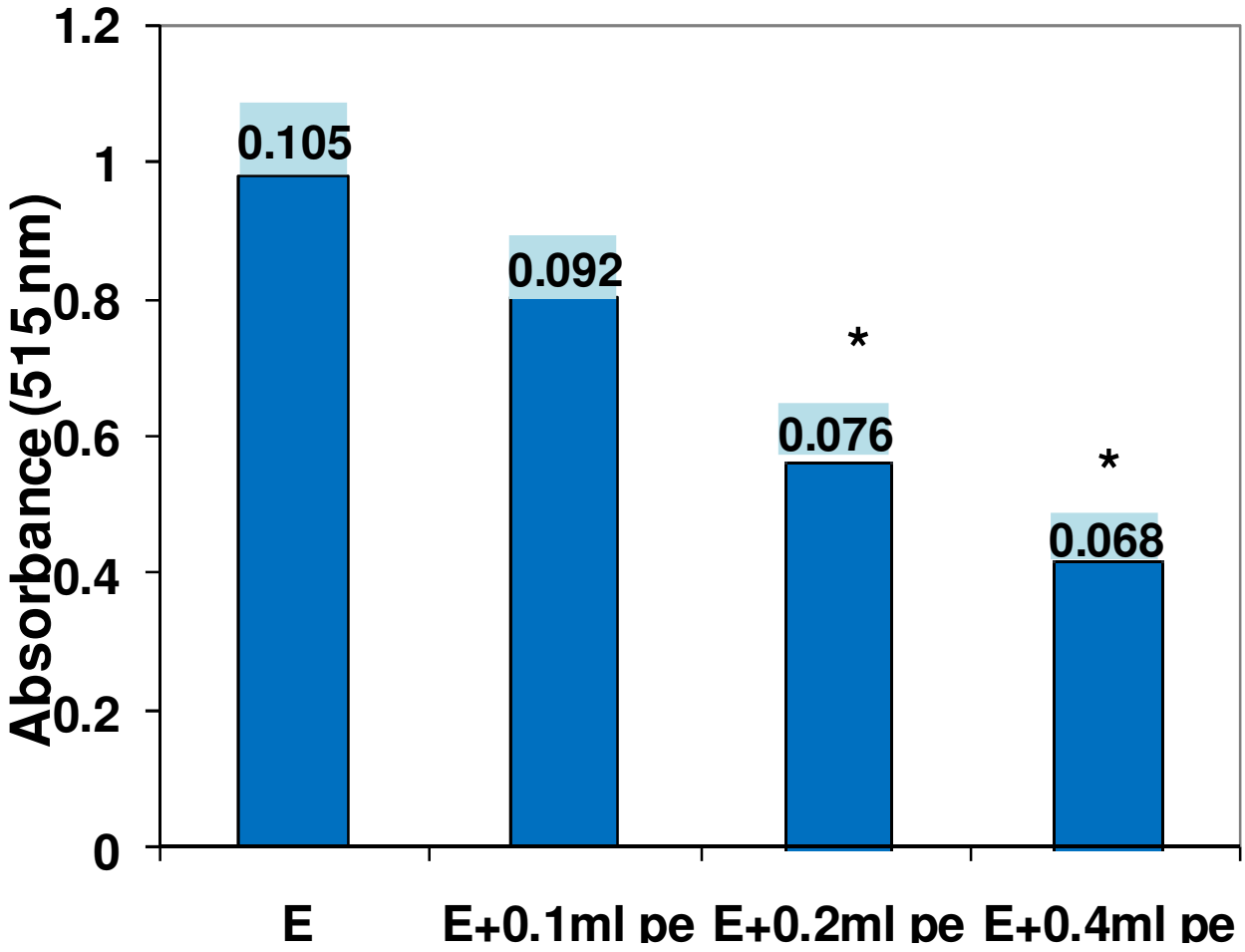

Figure 2. Antioxidant activity of different concentration from *Solanum melongena* peduncles extracts against free radicals generated *in vitro* by electrolysis (E) of a physiological buffer solution. 1 ml of the aqueous solution (pe) contain 98 mg of powder extract Values represent means ± SD of 6 experiments after 5 minutes electrolysis, * $p<0.05$ versus control (E).

system from 0.98 for the electrolyzed buffer alone (E) to 0.44 in presence of saliva from healthy volunteers (n = 10, $p<0.05$); while the saliva from patients with periodontal diseases showed a decreased absorbance to only 0.6. After 3 months of mouth wash with peduncles extracts (PDpe), the antioxidant activity of saliva was significantly improved. There was no significant improvement, when using the placebo (PDpl).

Total Antioxidant Activity (TAA) as well as glutathione activities were measured in the saliva of both healthy individuals and patients with periodontal diseases. Table 1 shows results similar to those obtained with electrolysis. Thus the two activities were significantly lower in patients with periodontal diseases (PD) compared to healthy volunteers (HV). While in treated group with peduncles extract TAA and glutathione were improved, no significant change was noted in the placebo group.

The clinical examination reported the pocket depth (PD), and the bleeding index (BI) parameters (Table 2). All these values were significantly ameliorated in the group that used peduncle extracts as compared to the placebo.

DISCUSSION

Periodontal diseases affect between 10 and 15% of people worldwide. It is a leading cause of tooth loss which develops as a result of bacterial infection from the build-up of the sticky, colorless bacterial plaque that continually forms on the surface of the teeth, especially in between the crevices. The inflammation that results from such infection destroys the attachment fibers and supporting bone that hold the teeth in the mouth (Papapanou, 1996).

Routine brushing, twice daily, and cleaning once interdentally has been the milestone of oral hygiene recommendations by dental professionals for decades. This regimen undoubtedly, has contributed to a better oral

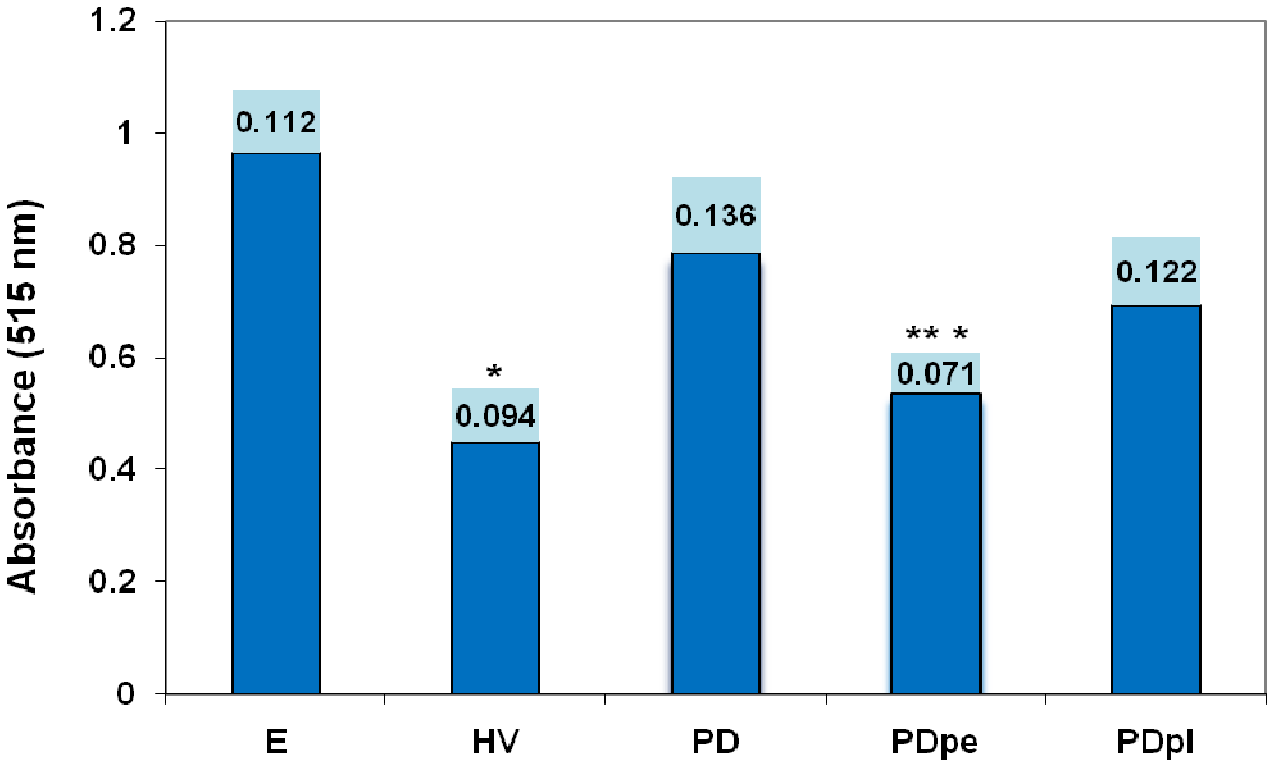

Figure 3. Antioxidant activity against free radicals generated by electrolysis (E) of saliva from 10 healthy volunteers (HV) and 20 patients with periodontal diseases (PD). Results from 10 of them after a mouth wash during 3 months with extracts of *Solanum melongena* peduncles (PDpe) or 10 with placebo (Pdpl), * $p<0.05$ versus E; ** $p<0.05$ versus PD.

health. However, when we consider contemporary incidence and prevalence of periodontal diseases, it becomes apparent that adjunctive methods of plaque and gingivitis control could be helpful for most patients.

Health care professional's recommendations should be based on clinically relevant scientific evidence. A number of published clinical studies support the recommendation of the adjunctive use of antiseptic mouth rinses. We designed our study to quantify specifically the expected incremental benefits derived from the adjunctive use of a natural extract from egg plant with antioxidant and anti-inflammatory properties (Sudheesh et al., 1999; Noda et al., 2000; Han et al., 2003) in patients who brush and floss as recommended. That is, in this long-term, three-months study, we aimed to determine whether brushing, flossing and rinsing with a natural product as mouth rinse were more effective than only brushing and flossing routinely in helping reduce periodontal diseases. In this context, some studies on experimental mouth rinse like Listerine permitted patients to perform their existing oral care regimens, which may have included daily interdental cleaning (Battino et al., 2002; Albert-Kiszely et al., 2007).

The results of our study give substantial evidence that the adjunctive use of aqueous extract from the peduncles of *S. melongena* provided a clinically significant and meaningful benefit in patients with gingival inflammation. This was apparent from the significant reduction in periodontal diseases against placebo of, Pocket Depth and Bleeding Index in patients who brush and floss regularly as a result of rinsing with this extract. Specific analysis concerning the improvement of the antioxidant properties of saliva after 3 months of mouth rinse in patients with periodontal diseases in which total antioxidant and glutathione activities were very low clearly demonstrated that free radicals are implicated at least partially in this disease (Lynch et al., 1997).

In most studies dealing with this subject, the antioxidant capacity of both the systemic (plasma) and local (crevicular fluid, saliva) samples were significantly lower in patients with periodontal diseases than in those with healthy teeth and gums. Glutathione levels, an evidence of the neutralization (scavenging) of free radicals were also low in those patients with periodontal diseases, but very high in those with healthy gum. This may be part of a defense strategy against bacterial invasion at exposed surfaces. In severe periodontitis, the immune cell response appeared to be imbalanced. The white cells became hyperactive, leading to increased inflammation and overproduction of oxygen free radicals; whereas, scavenger (glutathione) levels are too low to cope with that situation (Moore et al., 1994; Han et al., 2003; Lynch et al., 1997; Mc Cord, 1993). *S. melongena*

Table 1.Total antioxidant activity (TAA) and the Glutathione activity of saliva collected from healthy individuals compared to that collected from patients with periodontal diseases (PD), before and after a mouth wash with extracts of *Solanum melongena* peduncles (pe) against placebo (pl) during 3 months, * p< 0.05 versus PD.

	Healthy	PD (before)	PD+ pl	PD + pe
TAA ($\mu M.L^{-1}$)	299 ± 28	255 ± 22	264 ± 32	289 ± 27 *
Glutathione ($\mu M.L^{-1}$)	3.8 ± 0.33	3.12 ± 0.21	3.14 ± 0.26	3.6 ± 0.22 *

Table 2. Mean ± SD of pocket depth and bleeding index parameters before and after treatment with peduncle extract (pe) or placebo (pl), * p< 0.05 versus pre-treatment**.**

Group	Pocket depth (mm)		Bleeding index (%)	
	pe	pl	pe	pl
Pre-treatment	5.18±0.59	5.12±0.6 5	98.5±9.88	95.51±10.5
Post-treatment	3.49±0.70*	4.31±0.53	35.3±14.7*	68.5±18.79

extracts with antioxidant and anti-inflammatory properties are able to reestablish this imbalance.

It is important to note that most of the patients were active smokers, it is obvious that *S. melongena* extracts may protect oral cavity by enhancing its antioxidant properties, and consequently the adverse effect of smoking was attenuated. This point needs to be more investigated (Zappacosta et al., 1999; Whitaker and Stommel, 2003).

Thus, we conclude that:

The saliva of patients with periodontal diseases possesses weaker antioxidant activity compared to the saliva of participants with healthy oral cavity.

Aqueous extracts from peduncles of S. *melongena* possess a protective effect against the periodontal diseases and improve the state of the gum due to their antioxidant and anti-inflammatory effect.

A better knowledge of regulatory mechanisms of the oxidant/ antioxidant system at the level of oral cavity is still necessary, so as to define the therapeutic strategies as well as the way of protection or prevention.

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