

## Healing Effects of Hydroalcoholic Extract of Guava (*Psidium guajava*) Leaf on Oral Mucositis Induced By 5-Fluorouracil Using Histopathologic and Tissue Antioxidative Markers Assessment in Male Dark Brown Rats

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### Original Research Article

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**Abstract:** Oral mucositis (OM) is a common inflammatory complication among cancerous patients as an adverse effect of chemotherapy and radiotherapy. The aim of this study is to evaluate the healing effects of hydroalcoholic extract of *Psidium Guajava* leaf on oral induced mucositis induced by 5-fluorouracil using histopathologic and tissue antioxidative markers assessment in male dark brown rats. In a prospective randomized double blind animal study, OM was induced in 64 male dark brown rats that allocated in 4 groups by 5-FU (60 mg/kg) on days 0, 5, and 10 of the study. The cheek pouch was scratched with a sterile needle on once daily on days 3 and 4. Starting from day 12, gel base, topical form and 600 mg/kg dietary form of hydroalcoholic extract of *Psidium Guajava* leaf were administered per day. Pouch histopathology score, superoxide dismutase, glutathione peroxidase, total antioxidant capacity were evaluated on day 14 and 18. DPPH scavenging activity and total phenolic content also were measured. Histopathology scores of mucositis were lower in the systemic and topical treatment groups than the gel base and control groups ( $P < 0.05$ ). Higher activities of SOD, GPX and TAC were detected in the topical and systemic treatment groups in comparison to the others ( $P < 0.05$ ). The extract was rich in total phenolic content as antioxidant. The use of extract of *Psidium Guajava* leave may be associated with reduced intensity of OM, increased concentration of SOD, GPX and TAC on induced OM in dark brown rats undergoing 5-FU consumption. This may be due to appropriate antioxidant activity and phenolic contents.

**Keywords:** Oral Mucositis, Rat, Guava leaf.

### INTRODUCTION

Oral mucosa is the first defense line against digested agents and often suffers histo-pathological and inflammatory injuries which can lead to Oral Mucositis (OM). Oral Mucositis is the painful inflammation and ulceration of the mucous membranes lining the oral cavity, usually as an adverse effect of chemo- and radiotherapy as cancer treatments. They can interfere with maturity, growth, and renewal rate of basal epithelial cells, change the normal turnover, and cause cell death. Indeed, OM is the result of inflammatory changes in the epithelial and sub-epithelial cells of the oral mucosa and is a debilitating toxicity of chemo-radiotherapy. One of the chemotherapeutic drugs that can induce OM is 5-fluorouracil (5-FU). 5-FU is a pyrimidine analog drug which is used in the treatment of cancer. 5-FU has been administered systemically for anal, breast, colorectal, esophageal, stomach, pancreatic, and skin cancers and topically for actinic keratosis and Bowen's disease. Despite the therapeutic effects of 5-FU, it can induce OM as an adverse effect

and this requires prevention or treatment. For over a decade, complementary and alternative medicine has been used in the treatment or prevention of several diseases. Among them, the use of traditional medicinal plants has a special scientific position. These plants or their active substances in the form of aqueous and hydroalcoholic extract or essential oil can be used topically or systemic. Management of OM via topical approaches provided patient acceptance but using systemic route can guarantee the exact dose of consumption. Although a previous study was performed about using dexamethasone as a chemical drug for prevention of OM [13], the use of medicinal plants has attracted more attention. Our previous studies demonstrated that some medicinal plants such as bilberry [14], St. John's wort [16], Marigold [15], and *Carum carvi* L. in both forms of topical and systemic administration could effectively intervene against the development of OM.

*Psidium guajava* L. (Myrtaceae), commonly known as guava, has long been used in folk medicines as a therapeutic agent for the treatment of a number of diseases, e.g., as an anti-inflammatory, for diabetes, rheumatic pain, hypertension, wounds, ulcers, and reducing fever. Over the last few decades, extracts of guava leaves have been heavily commercialized in Taiwan, Japan, China, and Korea, and these extracts are commonly taken as dietary supplements showing various pharmacological effects. The main constituents of guava leaf extract are a variety of polyphenolics, flavonoids, and triterpenoids. However, regarding OM, previous reports mostly focused on the other species rather than *P. guajava* and no studies were performed on this species.

The best way for measuring the oxidative condition in the inflamed tissues and in response to any treatment is measuring the oxidative indices and also antioxidative components such as Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx) and Total Antioxidant Capacity (TAC).

Therefore, the aims of this study are to evaluate and compare the healing effects of hydroalcoholic extract of *psidium guajava* in topical form and dietary on 5-FU-induced OM in golden hamster by histopathology and antioxidative, and assessments. Also, the hydroalcoholic extract of *psidium guajava* is analyzed phytochemically to find potential bioactive substances.

## MATERIALS AND METHODS

### *Type of study and study population*

This study was approved by the Research and Ethics Committee of RKDF Dental College & Research Centre, Bhopal, and all relevant considerations about animal rights were taken into this study. This randomized animal trial was performed in RKDF Medical College Hospital & Research Centre, Bhopal, Madhya Pradesh, India under 64 dark brown rats with weight of 80-130 gram and age of 6-8 weeks old.

### *Animals and housing*

The animals were housed in standard cages under a 12-h light cycle (lights on at 7:00 pm) with an ambient temperature of  $22 \pm 2^\circ\text{C}$ , and  $55 \pm 5\%$  relative humidity. Rats were given a 3-day acclimation period with an access to normal chow and water ad libitum before the experiment. During their stay in the respective housing conditions, they were removed from their cages every 3 days for cleaning the cages and renewing their food and water supply. During the experiment, access to standard laboratory diet and water was allowed ad libitum.

### *Preparation of hydro alcoholic extracts*

The leaves of *Psidium Guajava* were collected from Bhopal, Madhya Pradesh, India. The plants were dried at room temperature and powdered in a grinder.

Aqueous ethanol (75%) was added to the powdered materials (500 g), and stirred for one hour. The mixture was kept at room temperature for 48 hours. Following filtration, ethanol was evaporated under reduced pressure at  $40^\circ\text{C}$ . The remained water extract was dried at oven temperature of  $50^\circ\text{C}$ .

### *Phytochemical Analysis*

#### **Determination of Total Phenolic Content**

The total phenolic content (TPC) of the *P. Guajava* extract was evaluated according to the modified Folin-Ciocalteu spectrophotometric method described previously by Waterhouse [1, 2]. The method involves the reduction of Folin Ciocalteu reagent by phenolic compounds. An aliquot of 40 mL solution of the 1 mg/ml extract was added to 3.16 mL distilled water and 200 mL Folin-Ciocalteu agents. The mixture was mixed well for 10 minutes. To this solution, 600 mL of a 0.25% sodium carbonate solution was added. The absorbance of the resulting solution was measured at 765 nm against the blank after 2 h incubation at room temperature using a UltraViolet-visible spectrophotometer. Quantitative measurements were done, based on a calibration curve of gallic acid as standard phenolic compound in methanol. The concentration of the total phenolic was expressed as milligrams of gallic acid equivalents per gram of dry extract (mg of GA/g of dE).

#### **Total Antioxidant Activity Assay**

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical that accepts an electron or hydrogen radical and converts into yellow- acity of DPPH is estimated by the decolorized diphenylpicyl hydrazine. The reduction capease in the absorbance at 517 nm induced by antioxidants. DPPH scavenging activity was measured by the spectrophotometric method according to Brand-Williams *et al.* [3] method (3). Briefly, in a 96-well micro plate, 270  $\mu\text{L}$  different concentration of plant extract or standard solution mixed with 20  $\mu\text{L}$  of (100 $\mu\text{M}$ ) DPPH solution dissolved in absolute methyl alcohol. After 30 minutes of incubation at room temperature, the absorbance of the sample was measured at 517 nm by a spectrophotometer. Quercetin was used as a positive control and the percentage of DPPH scavenging was calculated for each sample based on the equation:

DPPH Scavenging activity (%) =  $[(A_0 - A_1) / A_0] * 100$   
“ $A_0$  is the absorbance of the control, and  $A_1$  is the absorbance of the extract. The antioxidant activity of the extract was expressed as IC<sub>50</sub>.”

#### **Chemotherapy induced OM**

Sixty four golden hamsters were randomly allocated into 4 groups (16 hamsters in each group). All animals received three intraperitoneal injections of 5-FU on days 0, 5, and 10 at a dose of 60 mg/kg with insulin needles following the protocol proposed by Sonis *et al.* [4]. To mimic the clinical effect of chronic

irritation, the cheek pouch mucosa was scratched superficially with the tip of an 18-gauge sterile needle after anesthetizing the area. Two horizontal linear scratches were made across the everted cheek pouch once daily on days 3 and 4.

#### **Experimental design**

The maximum severity of OM was seen on day 12 (base on clinical examination) and the treatment thus was started on this day. The Group 1 of rats served as controls and didn't receive any treatment; Group 2 received gel base used in preparation of the topical gel daily; Group 3 were treated daily with topical gel 20% of hydroalcoholic extract of *Psidium Guajava* leaf on the cheek pouch mucosa; treatments were applied on both sides once a day (morning) and the affected area was completely covered. To make sure that gel was not swallowed, animals weren't allowed to eat or drink for 45 minutes after the drug applications. Group 4 were treated daily with dietary hydroalcoholic extract of *Psidium Guajava* leaf at oral dose of 600 mg/kg. Eight rats from each group were randomly selected for euthanizing on days 14 and 18, respectively. At the time of sacrifice, they were anesthetized with 100 mg/kg ketamine 10% (Alfasan, Netherlands) and 10 mg/kg xylocaine 2% (Alfasan, Netherlands). The both side cheek pouch tissue of hamsters in all groups were harvested on day 14 and 18. Tissue obtained from the right side pouch was proceed and stained with hematoxylin-eosin according to previous reported procedure. Tissues obtained from the left side pouch were stored under liquid nitrogen until antioxidant measurement.

#### **Histopathological study**

In each animal, the buccal mucosa was excised, fixed in 10% formaldehyde, and sent to the laboratory of the Department of Oral and Maxillofacial Pathology at RKDF Dental College & Research Centre for histopathologic examination. Specimens were grossly examined for a site with the most remarkable clinical changes. Multiple sections were cut from paraffin-embedded specimens and stained with hematoxylin-eosin for examination by an oral and maxillofacial pathologist [5-7]. The specimens were received scores of 0 to 3 based on a valid OM microscopic scoring system described by Lima *et al* as follows[8]:

Score0: normal epithelium and connective tissue without vasodilatation; absent or mild inflammatory infiltrate; absence of bleeding, ulcers and abscesses.

Score1: mild vascular hyperemia; areas of reepithelialization; mild inflammatory infiltrate with a prevalence of mononuclear cells; no hemorrhagic areas, ulcerations or abscesses.

Score2: moderate vascular redness; areas of epithelial degeneration; inflammatory infiltration with

prevalence of neutrophils; hemorrhagic areas, edema and occasional ulcerations; absence of abscesses.

Score3: severe hyperemia and vascular vasodilatation; inflammatory infiltration.

#### **Tissue Sample**

Samples obtained from the left cheeks were separated from skin and muscle layers. A certain amount (1.5 ml) of PBS buffer (pH= 7.4) was added to 0.5 g mocusa and the mixture was homogenized thoroughly by hand or homogenizer. Then samples were centrifuged (at 4000-6000 RPM) for approximately 20 minutes. The supernatants were collected carefully and freezed at -20 °C. Samples should be aliquoted and kept one for examination and freezed the others for later use.

#### **Superoxide Dismutase (SOD) assay**

Total SOD activity was evaluated with SOD detection kit (ZellBio GmnH, Germany-CAT No. ZB-SOD-96A) according to the manufacturer's instructions. This kit can be used for SOD activity determination in the range of 5–100 U/mL with 1 U/mL sensitivity. The role of SOD is to accelerate the dismutation of the toxic superoxide (-O<sub>2</sub>) produced during oxidative energy processes to hydrogen peroxide and molecular oxygen. In this assay, SOD activity unit was considered as the amount of the sample that catalyzes decomposition of 1 μmol of O<sub>2</sub>- to H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub> in 1 minute. The final activity of SOD was determined calorimetrically at 420 nm. Finally the SOD levels were expressed as U/g wet tissue.

#### **Glutathione Peroxidase (GPx) assay**

The blood activity of GPx was evaluated with GPx detection Biorex kit (BioRexfars-Product code: BXC0551) based on Paglia and Valentine's (1967) method, according to the manufacturer's instructions. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. GPx catalyzes the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP<sup>+</sup>. The decrease in absorbance at 340 nm against a blank was measured spectrophotometry. One unit (U) of GPx activity was defined as the amount of enzyme that converts 1 μmol of NADPH to NADP<sup>+</sup> per minute. The GPx activity was expressed as unit per liter (U/g).

#### **Total Antioxidant Capacity (TAC) assay**

Total Antioxidant Capacity was evaluated with TAC detection kit following the manufacturer's protocol (ZellBio GmnH, Germany-CAT No.ZB-TAC-96A) on the basis of the oxidation reduction colorimetric assay at a wavelength of 490 nm. TAC level was considered as the amount of antioxidant in the

sample that was compared with ascorbic acid action as a standard. This method can determine TAC with 0.1 mM sensitivity (100µmol/L). Concentration of antioxidant agents in the samples were reported as mM.

**Statistical analysis**

Data were expressed as the median and mean ± SD. Statistical analysis was performed by the Kruskal-Wallis and Mann-Whitney tests using SPSS® statistical software, version 21.0 (SPSS Inc., Chicago, IL, USA) for Windows®. Differences were considered significant when *P* value <0.05.

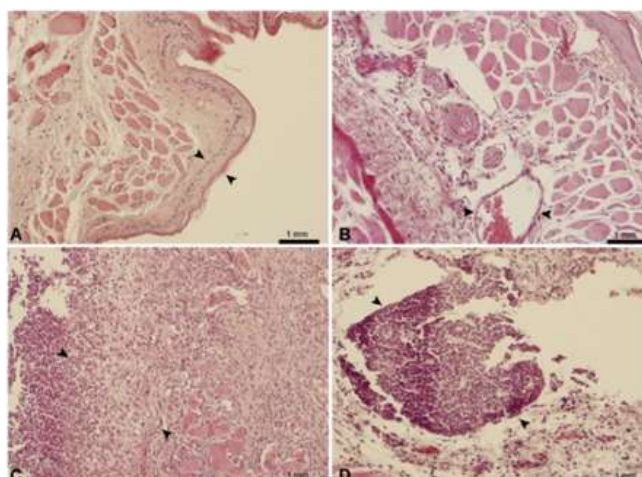
**RESULTS**

**DPPH Scavenging activity and TPC**

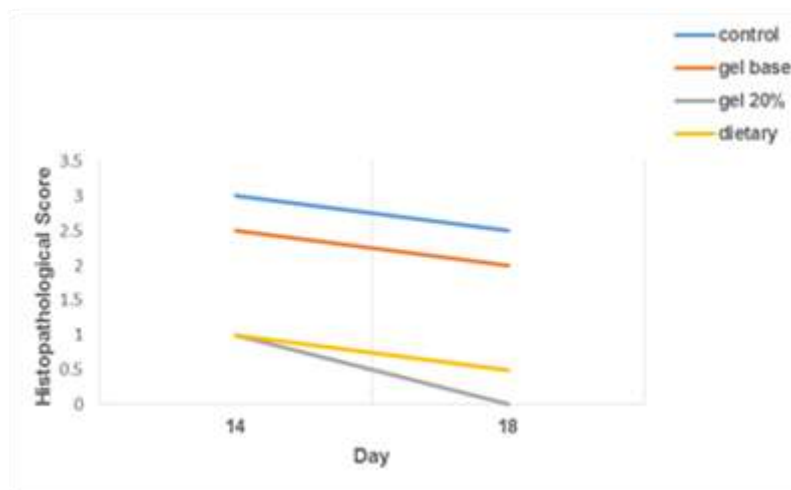
In this study, the antioxidant capacity of hydroalcoholic extract of *Psidium Guajava* was evaluated. Hydroalcoholic extract of *P.guajava* possessed the reasonable DPPH scavenging activity (IC50 =172 ± 3.592 ng/ml). TPC was 252.67±2.71 mg GAE/g of DE.

**Histopathology evaluation**

There was no histopathologic score (HPS) difference on days 14 and 18 in all four groups (control, gel base ,topical gel and dietary)(*P*=0.721, *P*=0.574, *P*=0.234, *P*=0.195). There was no significant difference in HPS between the gel base and control groups on days 14 and 18 (*P*=0.721, *P*=0.574), but significant decreases were seen in topical gel and systemic treatment groups compared to the gel base and control groups on both days (*P*<0.05). These declines indicating less severe inflammation in the topical gel and systemic treatment groups. Epithelialization and wound healing were detected in the topical gel and systemic treatment groups, whereas extensive infiltration of inflammatory cells, hemorrhage and ulcer were observed in the control and gel base groups. Topical gel and systemic treatment groups, also had no significant difference in HPS on days 14 and 18(*P*=0.195,*P*=0.382),but the topical gel group *P* value was lower than systemic treatment group, so we can understand the topical gel is more potent to decrease the inflammation.



**Fig-1: Evaluation of the healing effect of *Psidium Guajava* L. administration by histopathological changes in oral mucositis (OM) induced by 5-fluorouracil in hamsters: Histopathological scoring: (A) (score 0), from group treated with topical 20% gel; (B) (score 1), from group treated with 600 mg kg<sup>-1</sup>P. Guajava hydroalcoholic extract; (C) (score 2) from the gel base group; (D) (score 3) from the control group. (H&E staining)**

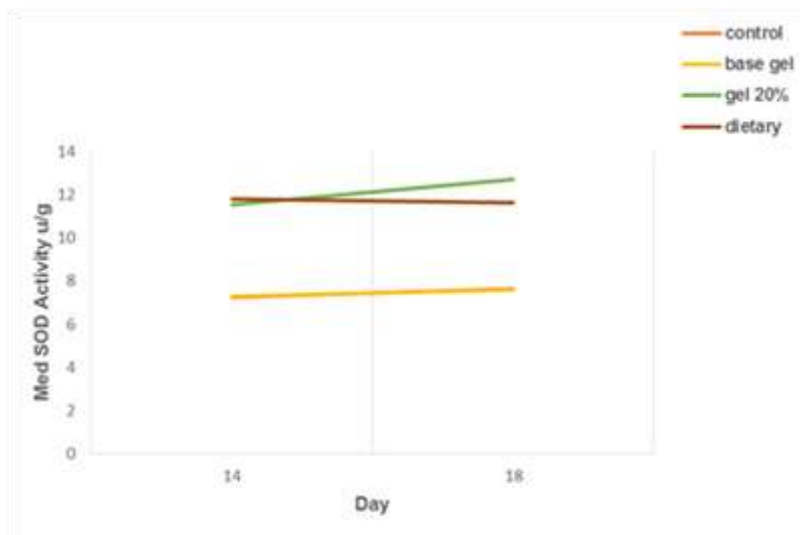


**Fig-2: The median comparison of histopathological score between four different groups in two days of sampling**

**Tissue SOD level assessment**

Measurement of tissue SOD concentrations in the study groups on days 14 and 18 are shown in Figure3. There were no significant differences in SOD concentrations between days 14 and 18 in all four groups (control, gel base, topical gel and dietary) (P=0.279, P=0.645, P=0.161, P=0.442). There was no significant difference in SOD concentration between the

gel base and control groups on days 14 and 18(P=0.878, P=0.645), but the SOD concentration was higher in topical gel and systemic treatment groups than the gel base and control groups on both days (P<0.05). No significant differences were detected between topical gel and systemic treatment groups on days 14 and 18(P=0.959, P=0.279).

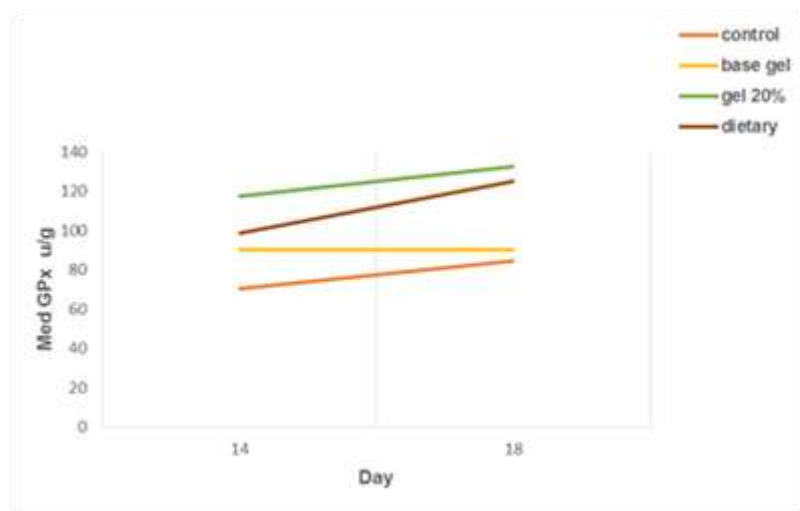


**Fig-3: The median comparison of SOD activity between four different groups in two days of sampling**

**Tissue GPx level assessment**

Measurement of tissue GPx concentrations in the study groups on days 14 and 18 are shown in Figure4. There were no significant differences in GPx concentrations between days 14 and 18 in all four groups (control, gel base, topical gel and dietary) (P=0.234, P=0.645, P=0.574, P=0.279). There was no significant difference in GPx concentration between the gel base and control groups on days 14 and 18(P=0.161, P=0.798). The GPx concentration was higher in topical gel group than the gel base and control

groups on both days(P<0.05). Also, the GPx concentration was higher in systemic treatment than control group on both days(P=0.028, P=0.005). There was no significant difference between systemic treatment and gel base group on day 14(P=0.195), but the GPx concentration was higher in systemic treatment group than the gel base group on day 18(P=0.007). No significant differences were detected between topical gel and systemic treatment groups on days 14 and 18(P=0.105, P=0.328).

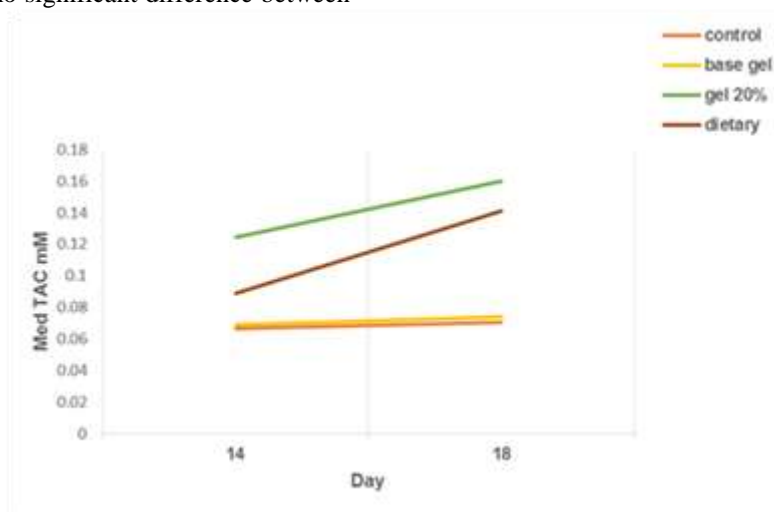


**Fig-4: The median comparison of Gpx concentration between four different groups in two days of sampling.**

**Tissue TAC level assessment**

Measurement of tissue TAC concentrations in the study groups on days 14 and 18 are shown in Figure 5. There were no significant differences in TAC concentrations between days 14 and 18 in three study groups (control, gel base and topical gel) ( $P=0.574$ ,  $P=0.798$ ,  $P=0.161$ ), but TAC concentrations in systemic treatment group on day 18 was higher than day 14 ( $P=0.038$ ). There was no significant difference between

the gel base and control groups on days 14 and 18 ( $P=0.442$ ,  $P=0.721$ ), but the TAC concentration was higher in topical gel and systemic treatment groups than the gel base and control groups on both days ( $P<0.05$ ). TAC concentration in topical gel group was higher than systemic treatment group on day 14 ( $P=0.049$ ), but no significant differences were detected between topical gel and systemic treatment.



Groups on day 18 ( $P=0.442$ ).

**Fig-5: The median comparison of TAC concentration between four different groups in two days of sampling**

**DISCUSSION**

Many patients with cancer are submitted to an initial therapy by radiotherapy (RT), surgery and chemotherapy (CT). RT is usually the treatment of choice in cases involving the head and neck, where the irradiation field involves the oral mucosa and salivary glands [9]. On the other hand, radiation is known as a producer of reactive oxygen species (ROS). When water, which constitutes around 80% of the cell, is exposed to ionizing radiation, decomposition occurs through which a variety of reactive oxygen species, such as the superoxide radical ( $O_2^-$ ), the hydrogen peroxide ( $H_2O_2$ ) and the hydroxyl radical ( $OH^-$ ) are generated. These ROS formed in cells contribute to radiation injury in cells. Although all respiring cells are equipped with protective enzymes such as SOD and GPx, increased oxidative stress in cells stemming from ionizing radiation may overwhelm the protective systems, leading to cell injury [10] and the injury is involved in the pathophysiology of many human diseases; for example heart disease, atherosclerosis, hepatotoxicity, inflammation, tumor promotion, and cancer [11]. Although, inflammatory diseases are currently treated with steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) [12], there are several reports about the healing and anti-inflammatory effects of medicinal plants in OM, as an inflammatory disease of oral mucosa, in human and animal models. Also, several investigations have been performed to elucidate the exact role of highly-specific molecules or

mechanisms underlying the OM development [13-18, 19-25].

The use of mouthwash containing 1% Chamomilla recutita extract in Braga FT *et al.* study associated with reduced incidence, intensity, and duration of mucositis in adult patients undergoes allogenic hematopoietic stem cell transplantation which suffered from OM [21]. Nader Tanideh *et al.* in 2013 conducted a study on assessing the potential of topical Calendula officinalis extract on the healing of oral mucositis induced by 5-fluorouracil (5-FU) in hamsters. Therefore this study showed that Calendula officinalis extract accelerated the healing of oral mucositis in hamsters and this effect related to the constituents of calendula such as triterpenoids and flavonoids that stimulate granulation and increase glycoprotein and collagen metabolism at the wound site [15]. In another study by Holmes TSV *et al.* in 2014, the effectiveness of Matricaria recutita Linn (chamomile) in the prevention and control of radiation-induced OM was evaluated. Their results showed that group with chamomile gel had the faster clinical improvement [23]. OmidKoochi-Hosseinabadi and colleagues on 2015 conducted a study on comparison of the therapeutic effects of the dietary and topical forms of Zizyphus jujuba extract on oral mucositis induced by 5-fluorouracil. The results showed that the use of Z. jujube hydroalcoholic extract in systemic and topical forms may be associated with reduced intensity of OM, diminished concentration of MDA, and increased

activity of MPO and SOD [16]. Mardani and colleagues in 2016 performed a study about the use of Hydroalcoholic extract of *Carum carvi* L. (CHE) in oral mucositis in golden hamster. Lower histopathology score and malondialdehyde level, higher reduced glutathione level and activities of myeloperoxidase were detected in 1000 and 500 mg kg<sup>-1</sup> per day topical CHE and control groups. The CHE was more potent against *Staphylococcus epidermidis* and *Streptococcus intermedius*. C-Terpinene (37.2%) was identified as the main constituent of essential oil [18]. In a study by Pahuja, it has been demonstrated that hydroalcoholic extract of *Z. jujube* can reverse oxidative stress induced by pentylentetrazole (PTZ) and electroshock [11].

In all previous reports, plants and their extracts had no prophylactic effect on the onset of OM, but it was proven to be effective in decreasing the severity of this condition. Also, it has been reported that the beneficial effects of these traditional plants may be due to polyphenols content which reviewed by Varoni *et al.* [24].

As far as we know, there is no experiment that has evaluated the anti-inflammatory and antioxidant activity of *Psidium Guajava* in OM. However, there are some reports about the beneficial effects of this traditional medicinal plant in other aspects. Hui-Yin Chen and his colleague conducted a study on the antioxidant activity and free radical-scavenging effects of extracts from guava leaves and dried fruit. The results indicated that 94.4–96.2% of linoleic acid oxidation was inhibited by the addition of guava leaf and guava tea extracts at a concentration of 100 µg/ml. The results also demonstrated that the scavenging effects of guava leaf extracts on ABTS<sup>+</sup> radicals and superoxide anion increased with increasing concentrations. The guava leaf extracts displayed a significant scavenging ability on the peroxy radicals [26]. The anti-inflammatory effects of an ethanolic extract of *Guava* (*Psidium guajava* L.) leaves were investigated by Mi Jang *et al.* in vitro and in vivo. Their results demonstrated that guava leaf extract (GLE) significantly inhibited lipopolysaccharide (LPS) - induced production of nitric oxide and prostaglandin E2 in a dose-dependent manner. GLE suppressed the expression and activity of both inducible nitric oxide synthase and cyclooxygenase-2 in part through the down regulation of ERK1/2 activation in RAW264.7 macrophages. Furthermore, GLE exhibited significant anti-inflammatory activity in 2 different animal models—Freund's complete adjuvant-induced hyperalgesia in the rat and LPS-induced endotoxic shock in mice. Also, 11 compounds were detected and identified in GLE, including phenolic acid and flavonoids. GLE showed the highest catechin content, followed by gallic acid, chlorogenic acid, and quercetin in decreasing order [27]. In a study by Chia-Yun Lin & Mei-Chin Yin, they analyzed the content of phenolic acids and flavonoids in extracts of guava fruit (*Psidium*

*guajava* L.), and examined the renal protective effects of guava aqueous extract (GAE) and ethanol extract (GEE) in diabetic mice. The findings support that guava fruit could protect kidney against diabetic progression via its anti-oxidative, anti-inflammatory and anti-glycative effects (106). Sarmistha Dutta and his colleague evaluated the effect of the ethanolic extract of the leaves of *Psidium guajava* (PGE) on experimentally induced colitis in animal models. The results indicate the ethanolic extract of *P. guajava* leaves showed significant amelioration of experimentally induced colitis, which may be attributed to its anti-inflammatory and anti-oxidant property [28].

According to these studies, Guava has been reported to contain numerous polyphenolic and other chemical compounds that have been shown to exhibit various pharmacological effects, including anti-inflammatory and antioxidant activities.

In our study, we compared the effects of two different drug forms of *Psidium Guajava* extract, topical gel 20% and 600 mg/kg dietary forms, on the healing of OM induced by 5-FU in golden male hamsters. Besides, we evaluated DPPH Scavenging activity and measured total phenolic content of hydroalcoholic extract of *P. guajava* leaves also histopathological score (HPS) and antioxidant markers such as SOD, GPx and TAC concentration in the groups on days 14 and 18. Our results demonstrated that both two drug forms can induce wound healing by elevating SOD, GPx and TAC activity and improving the histopathology features. Also, the topical gel was approximately more potent than systemic dietary form. Our results also indicated that guava leaves possess a reasonable DPPH scavenging activity and furthermore rich in phenolic content. Guava leaves anti-inflammatory activity might be due to an increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation. The anti-inflammatory activity of the extract may be due to the presence of certain polar constituents such as flavonoids that might be involved in the inhibition of prostaglandin synthetase.

It has been also demonstrated that plant polyphenols such as flavonoids are a most important active natural products possessing antioxidant activity. The flavonoids which contain hydroxyl groups are responsible for the radical scavenging or chelating effects. These agents accompanying with several endogenous antioxidants enzymes included SOD, GPx and TAC may play an important role in maximum protection against oxidative stress induced by high level of reactive oxygen species (ROS) in the body. On the other hand, phenolic compounds are a class of antioxidant agents which act as free radical terminators. According to our investigation, the high contents of the phenolic phytochemicals in *Psidium Guajava* and its antioxidant activity can support its high radical scavenging activity and following protective effect in

radiotherapy- induced OM. Initiation phase of radiotherapy or chemotherapy-induced injury is a critical first step in the development of mucositis in which clonogenic cell death and the production of ROS by injured cells are the most dominant components. Initiation phase is a rate-limiting step and by delaying or stopping it, it can be prevented or minimized by regimen-related injury. Considering the antioxidant properties of guava, it may act against ROS and prevents or delays the initiation phase. Our results are in agreement with those previous findings and demonstrated that hydroalcoholic extract of Psidium Guajava leaves possesses healing effects on experimental induced OM due to polyphenolic contents. These protective effects are seen in the groups receiving systemic and topical forms of extract.

### CONCLUSION

According to the findings of present study, Psidium Guajava might be effective agent in treatment of Oral Mucositis. Phenolic compounds appear to be responsible for the antioxidant activity and healing effect of guava extracts. However, further studies must be performed on the finding of its active anti-inflammatory and antioxidative components in different diseases and clinical trials. The steriological evaluation of guava extract on different diseases is recommended.

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