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Effect of *Tagetes erecta* flower and leaf extract gel on oxidative stress and antioxidant levels in oral ulcer condition

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ABSTRACT

An oral ulcer is described by repeated or painful oral aphthae, in which oxidative stress apparently leads to its pathogenesis. The present study has been undertaken to evaluate the effects of fresh leaves and flowers of *Tagetes erecta* gel on oxidative stress and antioxidant levels on oral ulcer models in albino Wistar rats. The oral ulcer was induced by incision wound model and then treated with the *Tagetes erecta* flower and leaf extract gel for 7 days. The antioxidant activities of catalase (CAT), superoxide dismutase (SOD), the levels of glutathione (GSH) and malondialdehyde (MDA) were measured in rat buccal mucosal tissues. The antiulcer effects of *Tagetes erecta* leaf and flower extracts (2.5% and 5%) gel were intercompared with the standard drug, Triamcinolone. Ulcer induced rats exhibited elevated levels of MDA and reduced activity of SOD, GSH and CAT when compared to the control rats. The non-equilibrium levels of antioxidant were observed in rat mucosal tissues indicate that body's antioxidant status was depleted with an oral ulcer. *Tagetes erecta* leaf and flower gel (5%) has been found to have higher significant antioxidant activity compared with ulcer induced rats by reducing the levels of MDA and by increasing the activities of SOD, GSH and CAT in response to oxidative stress due to induction of oral ulcers in a rat model. The findings thus justify the traditional use of *Tagetes erecta* to heal oral ulcer by increasing the antioxidant status and decreasing the free radical production.

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INTRODUCTION

Oral ulcer aetiology is largely unidentified, though various aspects are proposed as probable causative means. These factors include trauma, nutritional factors, microbial factors, immunologic factors, psychosocial stress, genetics, hormonal changes and allergy (Akintoye and Greenberg, 2014). These factors can interrupt the oxidant-antioxidant equilibrium of the person and thereby, activate the free radical formation.

Oxidative stress is the disproportion between the

number of reactive oxygen species (ROS) inside the cell and the capability of the cell to eliminate these free radicals and reactive peroxides. The molecular targets of ROS comprise unsaturated proteins, carbohydrates, phospholipid and nucleic acids (DNA or RNA); ROS can thus compromise cell structure and viability though there are studies on oxidative stress (OS) in oral ulcer (Momen-Beitollahi *et al.*, 2010; Bagan *et al.*, 2014). The oxidative stress (OS) takes place as a result of increased concentration of free radicals that suppress the activities of the immune system, which leads to cellular damage. To counteract this OS, mammalian cells have the antioxidant system that comprises both enzymatic and non-enzymatic effects. The enzymatic antioxidants include SOD, Catalase (CAT) and GSHPx; and the non-enzymatic antioxidants include vitamins E, A, C, UA, melatonin and glutathione (Saxena, 2011).

It has also been shown that essential oil from *Tagetes erecta* at the dose level possesses substantial antioxidant effect less than α-tocopherol (Lim, 2014). The principal antioxidant compound existing in the *Tagetes erecta* are lutein esters, tocopherol, β-tocopherol, γ-tocopherol and δ- tocopherol (Gong *et al.*, 2011). Lutein, a natural antioxidant, is the major pigment present in Tagetes species (Pratheesh *et al.*, 2009; Dixit *et al.*, 2013). *Tagetes erecta* flowers, rich in the carotenoid lutein, decreases inflammatory cytokine secretion and decreases the OS in an animal model of oral ulcer (Meurer *et al.*, 2019).

Based on the above findings, however, it is evident that ROS/antioxidant levels are imbalanced in the experimentally oral ulcer induced rats (Avci *et al.*, 2014; Iannitti *et al.*, 2012). Therefore, the current study has been commenced to scrutinize the effect of leaf and flower of *T.erecta* extract gel on oxidative stress and antioxidant levels in oral ulcer induced rat model.

MATERIALS AND METHODS

Collection and preparation of the sample

The whole plant of *Tagetes erecta* was obtained from the Tamil Nadu Agricultural University botanical garden, Coimbatore. The plant was recognized and documented by BSI, the Botanical Survey of India, and a voucher specimen was dropped with a voucher specimen sample No. BSI/WRC/Tech./2012/431 The dried clean petals and leaves were made into powder form using an electric mixer separately.

Extraction Procedure

The fresh plant petal and leaf part was shade dried for 20 days. A substantial quantity of dry flower petals was extracted using hydro alcohol at temp—40-60^{0}C by means of cold maceration. The hydro-alcoholic extract was filtered and concentrated to a dry mass using a rotary evaporator. A deep reddish-brown viscous residue formed having distinctive odour. The weight of the dried mass was measured. The yield was anticipated to be around 10%. Moreover, the solvents were vaporized to dryness by means of vacuum oven. The extract was placed inside a sterile glass bottle separately.

Formulation of the therapeutic gel

A water-soluble base like carbopol having methylparaben and propylparaben was taken as a base for leaf and flower extracts individually. 2.5 g of the extract was taken and mixed vigorously with the gel base containing Carbopol: 2.5g, propylparaben: 0.10gms, methylparaben: 0.01g, and distilled water: 97.50ml. The resultant mixture was Tagetes gel, 2.5%.

Experimental animals

Albino Wistar rats with a mass of 200-250 g were procured and lodged in ordinary cages at room temperature and were given food and water *ad libitum.* The methodology was accepted by the institutional animal ethics committee and animals were sustained under ordinary environments in an animal house permitted by CPCSEA (1559/PO/E/S/11/CPCSEA).

Incisional wound model

Before inflicting the wound, the rats were sedated using ether anaesthesia. The mouth was opened using mouth clamps and right buccal mucosa cleaned with chlorhexidine and marked. An incision of about 1cm was created using sterile BP blade on the right buccal mucosa in light ether anaesthesia at the sterile condition and observed all through the study and then the animals were housed individually.

Experimental grouping

Animals were classified into 7 groups, each having six animals. Group 1 rats served as normal controls. Group 2 served as ulcer induced rats, Group 3 and Group 4 rats were given 2.5% and 5% leaf extracts gel of *Tagetes erecta* respectively. Similarly, Group 5 and Group 6 rats were given 2.5% and 5% of plant flower extracts gel. Finally, the seventh group served as triamcinolone, the standard treated rats. The animals in all subgroups were treated twice daily at the same time. A cotton swab dipped inappropriate drug was applied topically to the ulcer from the second day for a period of 7days. In Tris - HCl buffer (0.1 M, 4°C, pH 7.4), the weighed buccal

mucosal tissues were homogenized using a Potter - Elvehjem homogenizer with a Teflon pestle run for 3 minutes at 600 rpm. It was then centrifuged at 3000 xg at 4°C for 10 minutes with Remi refrigerated centrifuge and taken for analyzing the antioxidant parameters.

Estimation of MDA

The tissue samples were kept in a hot water bath for 15 min. 1 mL of trichloroacetic acid and TCA-2-thiobarbituric acid (TBA)–HCl reagent was added to the sample. The reaction mixture was cooled and centrifuged. The supernatant was taken and the optical density of the pink colour created was read at an absorbance of 535 nm. The MDA concentration in the sample was observed by plotting the absorbance obtained against the standard graph. The optical density is directly proportional to the MDA concentration in the given sample. The concentration is measured in nmol/gram protein of the sample.

Assay of SOD activity

The SOD effect was measured conferring to McCord and Fridovich (1969). Solution A was made by mixing 100 ml of 50 mM PBS (pH 7.4) and 0.1 mM EDTA and 2 μM cytochrome c with 10 ml 0.001 N NaOH solution including 5 μM xanthine. Solution B was prepared by mixing 0.2 U xanthine oxidase/ml and 0.1mM EDTA. Fifty microliters of test sample were mixed with 2.9 ml of solution A tracked by 50 μl of solution B. Absorbance was measured at 550 nm. A blank was run with fifty microliters of ultra-pure water. SOD levels were expressed as U/mg protein with reference to the effect of a standard sample of bovine copper-zinc SOD under similar conditions.

Estimation of Catalase activity

The activity of CAT was measured in samples conferring to the procedure of Cimen *et al*. (2003). H_2O_2 decomposition was monitored at 240 nm by a spectrophotometer. The specific effect was resolute as micromole of substrate H_2O_2 decomposed in one minute per milligram of protein (U/mg protein).

Estimation of GSH

The tissue homogenates (prepared with pH of 7.4 in 0.1 mol/L phosphate buffer) was mixed with EDTA (0.02 mol/L) and the test tubes were kept in an ice bath for about 10 minutes to precipitate the tissue proteins. Then 0.5 mL trichloroacetic acid (50%) was added to every test tube having distilled water (2.0 mL) and kept in an ice bath for 10-15 minutes. The supernatants (1 mL) of each sample was transferred into another set of fresh test tubes and Tris-HCl of 2.0 mL (0.15 mol/L) and Dithio-bis of 50 μL (2-nitrobenzoic acid (6 mmol/L) were added and mixed vigorously. The absorption was measured at 412 nm within 2 minutes of the final step against blank. In a similar way, the blank was prepared without tissue homogenate. In the standard, instead of tissue homogenate, the GSH was added. In standard, 40-200 μg of concentrations were used to construct a standard curve of GSH. The GSH in the tissue homogenates was measured by using the linear regression equation and the concentration was measured in terms of U/g protein.

Statistical Analysis

The data were measured as the Mean$\pm$ SEM for each group. Statistical differences were assessed using a one-way analysis of variance (ANOVA). Results were measured to be statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Oxidative stress (OS) takes place when the concentrations of ROS rise above physiologic values, resulting in cellular damage (Saxena, 2011). Various antioxidant systems are produced by the body for ROS detoxification. This disparity between the free radicals and antioxidants are supposed to cause many inflammatory pathologies (Al-Snafi, 2017). Valuation of the levels of antioxidant enzyme activities such as those of CAT, SOD, and glutathione help to measure the antioxidant status of the extract (Cimen *et al.*, 2003; Babaee *et al.*, 2016).

MDA is a suitable biomarker of endogenous DNA damage. Reactive oxygen metabolites result in destruction and injury to cell membranes by lipid peroxidation, which results from the oxidation of membrane-associated polyunsaturated fatty acids of phospholipids. MDA is the principal end product of polyunsaturated fatty acid peroxidation and is a noble marker of free radical arbitrated oxidative stress and damage (Tavangar *et al.*, 2014).

Primary defense conflicting ROS is by catalytic deduction of ROS via antioxidant enzymes. SODs are metalloenzyme family initiated in all aerobic organisms and are the primary enzymes to be involved in antioxidant defense. They catalyse the dismutation of superoxide to hydrogen peroxide, three forms of SODs are present in mammalian tissues, each with a particular subcellular location and dissimilar tissue distribution (Zhang *et al.*, 2017). During OS, the cell reacts to reactive oxygen metabolites with SOD. SOD protects the cell from damage instigated by superoxide (O_2^-) and hydroxyl radical, release H_2O_2 in the process. Whereas SOD drops the steady-state level of O_2^-, catalase and peroxidases do the same for H_2O_2 (Jesija, 2017).

In the present study MDA, the oxidative stress marker was found higher among the ulcer induced

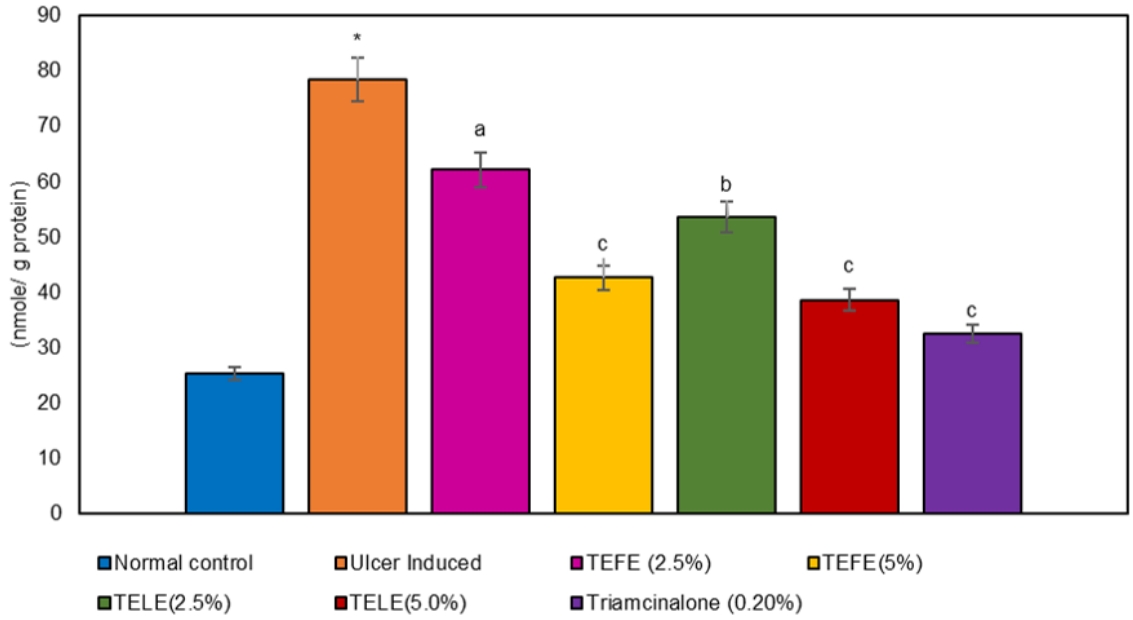

Figure 1: Levels of Malondialdehyde in oral ulcer model upon Tagetes erecta leaf and flower extract gel treatment. Results were measured as Mean ± SEM (n=6). ***p<0.001 statistically significant as compared with the normal control group. [a]p <0.05; [b]p<0.01; [c]p<0.001 statistically significant as compared with ulcer induced group. TELE - Tagetes erecta leaf extract, TEFE - Tagetes erecta flower extract

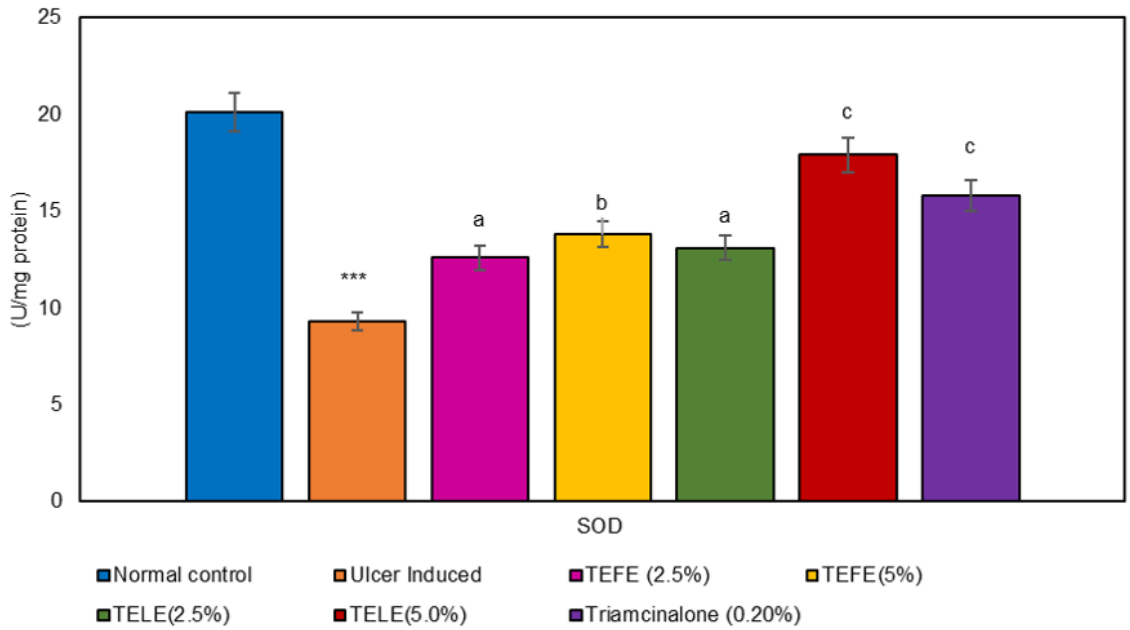

Figure 2: Activities of Superoxide dismutase in oral ulcer model upon Tagetes erecta leaf and flower extract gel treatment. Results were measured as Mean ± SEM (n=6). ***p<0.001statistically significant as compared with the normal control group. [a]p <0.05; [b]p<0.01; [c]p<0.001 statistically significant as compared with ulcer induced group.

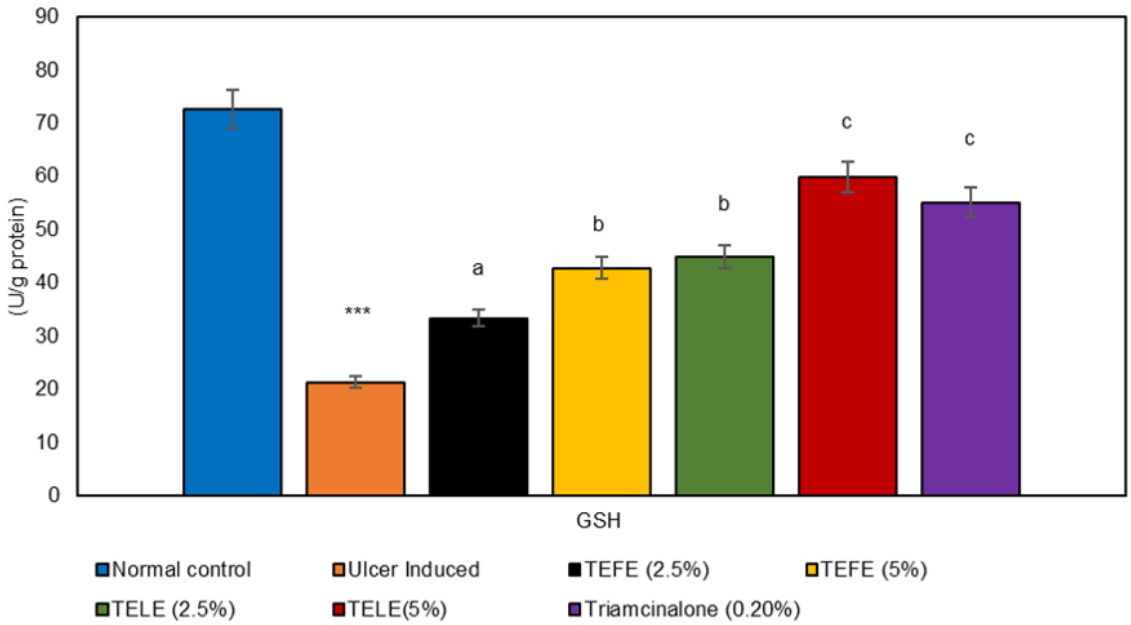

Figure 3: Levels of glutathione in oral ulcer model upon Tagetes erecta leaf and flower extract gel treatment. Results were measured as Mean ± SEM (n=6).* p<0.001 statistically significant as compared with the normal control group. [a] p <0.05; [b] p<0.01; [c] p<0.001 statistically significant as compared with ulcer induced group.**

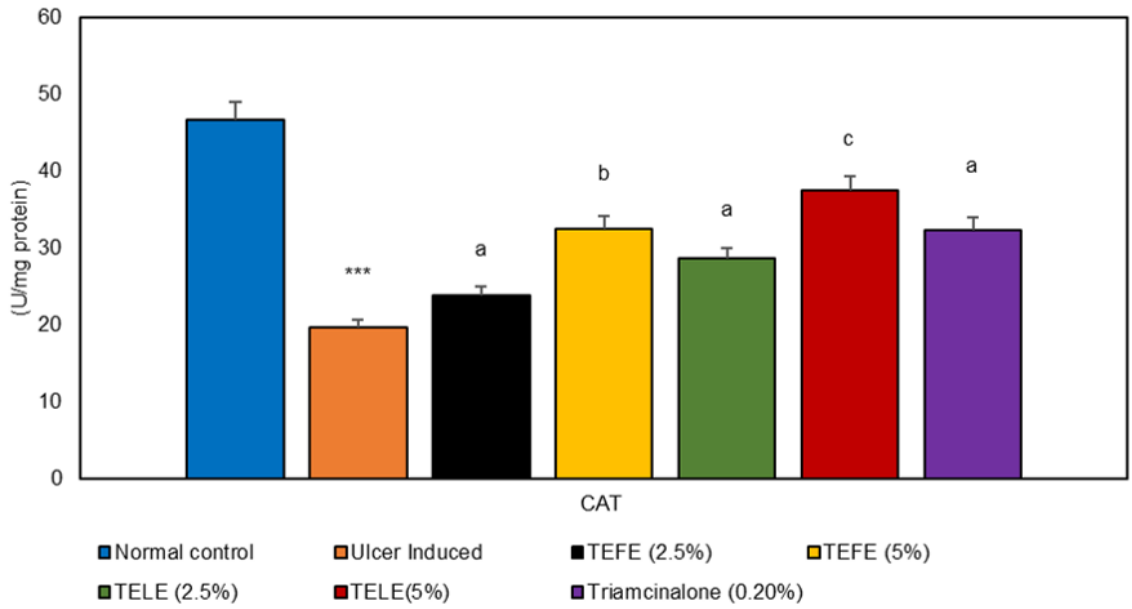

Figure 4: Activities of catalase in oral ulcer modelupon Tagetes erecta leaf and flower extract gel treatment. Results were measured as Mean ± SEM (n=6). *** p<0.001 statistically significant as compared with the normal control group. [a] p <0.05; [b] p<0.01; [c] p<0.001statistically significant as compared with ulcer induced group.

group when compared to the control rats. Analysis of antioxidants showed significantly decreased effects of SOD, glutathione and catalase in the ulcer induced group when compared to the control ones. Flower extracts (5%) of Tagetes erecta was observed to have a highly significantly lower level of MDA when compared among the treatment groups (Figure 1). The activities of enzymatic antioxidants SOD, CAT, and non-enzymatic antioxidant, GSH, were found more increased in the rat's given leaf extract (5%), flower extract (5%) than leaf extract (2.5%) flower extract (5%) of *Tagetes erecta* (Figure 2, Figure 3 and Figure 4) when compared to the ulcer induced groups.

MDA was reduced by *Tagetes erecta* flower extract as well as by *T. erecta* leaf extract, though the effect of Triamcinolone was less significant. The previous study proved that *T. erecta* acts as an antioxidant in the buccal mucosa tissue (Figure 1). The redox status of the cells is of significance to find the cellular anti-oxidant defense (Gümüş *et al.*, 2016). *Tagetes erecta* might be exerting an additional antioxidant activity by preventing histamine-induced damage through other receptors.

Studies showed that *Tagetes erecta* a noticeable effect in scavenging superoxide radical because of their higher total phenolics and flavonoids contents (Siddhu and Saxena, 2018). The reduced activity of CAT, SOD, and GSH in tissues of ulcer induced group might be because of either the feedback inhibition or oxidative inactivation of enzyme protein because of the excess generation of ROS. H_2O_2 generation may also result in the inactivation of this enzyme, but SOD, CAT and GSH levels were increased in *T. erecta* flower and leaf gel treatment (Figure 2, Figure 3 and Figure 4). This activity can be attributed to a donation of hydrogen, to an electron reduction or direct scavenger effects. During the detoxification of *increased* H_2O_2 by glutathione, consumption of chemically abridged *GSH* also rises (Jesija, 2017). Reka and Anuradha (2011) stated that tissue GSH levels are considerably abridged by oxidative stress and proposed that antioxidant defense impairment mechanisms could permit enhanced free radical prompted tissue damage.

The data presented here show that *T. erecta* extracts have significant antioxidant activity against oral ulcer may be used as an alternative to the synthetic antioxidants (Miricescu *et al.*, 2011). To conclude, the decrease in MDA and the increase in SOD, GSH and CAT produced by *Tagetes erecta* leaf extracts gel comparatively highly significant as compared with flower extract gels in oral ulcer which could be a mechanism of action for its antiulcer activity.

CONCLUSION

Thus, the present study offers a strong impact on growing the investigations of natural antioxidants. This article revealed that *T. erecta* is a significant medicinal plant for oral ulcer and a potent antioxidant. Further studies should be carried out for isolation and characterization of bioactive compounds from *Tagetes erecta* which independently or in adjunct are responsible for showing antioxidant activity for the beneficial protection against this oral ulcer.

Conflict of Interest

None.

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