

Evaluation The effect of Chicory and Malva Leaves as Antioxidant and Anti-Inflammatory

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ABSTRACT

Chicory (*Cichorium intybus*) and Malva (*Malva parviflora*) plants have been used as a traditional medicinal plants . This study aimed to evaluate the total phenols and flavonoids of chicory and malva leaves . Qualitative HPLC profile of the chicory and malva leaves was performed using HPLC. Antioxidant activity was determined by three methods (hydroxyl radical, superoxide anion radical DPPH^{*} and ABTS+ cation radical). The anti-inflammatory activity of aqueous extracts of these plants was assessed using carrageenan-induced paw edema was studied. The results showed that chicory and malva leaves can represent a good sources of phytochemicals in terms of total phenolics and flavonoids as well as the ability of these phytochemicals to exert antioxidant and anti-inflammatory activity of aqueous extracts of these plants. Results concluded that the consumption of chicory and malva leaves may be beneficial to human health by participating in the antioxidant defense system against free radical generation and anti-inflammatory

Keywords: Chicory leaves , Malva leaves , Antioxidant Activity , Anti-inflammatory activity

INTRODUCTION

Oxidative stress is involved in the pathology of cancer, malaria, arteriosclerosis and rheumatoid arthritis, may be play a role in neurodegenerative diseases and ageing processes (**Rammal *et al.*, 2008 a, Rammal *et al.*, 2008 b**). Plants have medicinal components that can be used as alternative remedies for many diseases. Plants and plant extracts are used by about three quarter of the world's population for their healthcare.

Inflammation is a pathophysiological response of mammalian tissues to a variety of hostile agents including infectious organisms, toxic chemical substances, physical injury or tumor growth leading to local accumulation of plasmic fluid and blood cells (**Sobota *et al.*, 2000**).

Kumara (2001) revealed that, plant based drugs in traditional medicine are being paid much attention because of their few side effects, cheapness and the fact that 80% of the world population still uses them. Epidemiological and in vitro studies suggest that foods containing phytochemicals such as phenolic compounds have potential protective effects against many diseases. Therefore, they may be used as anti-mutagenic, antibacterial, antiviral and anti-inflammatory agents (**Senevirathne *et al.*, 2006**). The Mediterranean diet, rich in fruit, vegetables, and fish, has been associated with a lowered incidence of disease and an overall improvement in health (**Trichopoulou *et al.*, 2003**).

Chicory (*Cichorium intybus L*) commonly known as chicory a member of the Asteraceae family and widely grown in Europe, Western Asia, Egypt, and North America. Historically, chicory was grown by the ancient Egyptians as a medicinal plant, vegetable crop and was occasionally used for animal forage. (Mulabagal *et al.*, 2009) . Chicory leaves, flowers, and roots can all be used, with the leaves and flowers being added to the salads also the roots being used in the coffee blends (Jancic *et al.*, 2017). Chicory leaves have a slightly bitter taste may be due to the presence of a bitter glycoside named cichorine, according to Zaman and Basar (2013) . Chicory leaves are a valuable therapeutic herb which contain important constituents such as caffeic acid derivatives, polyphenol and flavonoids Kocsis (2003) . Chicory roots and leaves contain inulin and fructo-oligosaccharides prebiotics, both of them are beneficial to human health Bais and Ravishankar (2001) . Furthermore, chicory leaves are good sources of phenols, vitamins A(as carotenoids) and C as well as potassium, calcium, and phosphorus (Mulabagal *et al.*, 2009). Chicory own excessive medicinal importance as it has inulin, coumarins, sesquiterpene lactones, alkaloids vitamins, chlorophyll pigments, unsaturated sterols, flavonoids, saponins, and tannins (Abbas *et al.* 2015). Chicory is found to be effective in rheumatic complaints, anti-cancer, anti-fungal , anti-malarial and anti-diabetic (Lante *et al.*, 2011). Cichorium species leaves have been used for centuries as part of traditional diet in the Mediterranean countries (as salads or cooked vegetable, and in meat dishes), while the roots are baked, ground, and used as a substitute for coffee and inulin source. chicory leaves extract could be used as a natural anti-inflammatory agent(Bayazid *et al.* .2020).

Malva parviflora L. (family Malvaceae) is herb native to Africa, Asia and Europe. Its common name is cheese weed. Pharmacologically, it has been reported to be antidiabetic (**Gutierrez, 2012**), antibacterial (**Shale et al., 2005**), antifungal (**Wang et al., 2001**). The plant does not have strong or exciting taste, but does make a pleasant addition to salads and can be cooked as a green. The young leaves of *Malva parviflora* that look like lettuce sound are processed as Greens in the west and used as rice stuffing (**Veshkurova et al., 2006**). Traditionally Malva is used for the treatment of liver injuries and inflammation pain (**Afolayan et al. 2010**). *M. parviflora* may be a natural source of antioxidants and thus useful as a therapeutic agents in the slowing of aging and in the relief of age-related and oxidative stress-related degenerative diseases (**Farhan et al., 2012**). *M. parviflora* has demonstrated antihypertensive, antioxidant, and anti-inflammatory effects attributed to oleanolic acid, tiliroside, and scopoletin (**Lagunas-Herrera et al. 2019**). **Martínez-Hernández et al. (2020)** reported that anti-inflammatory and anti-rheumatoid activities of a fraction rich in sterols from *Malva parviflora*. Moreover, its phenolic compounds have multiple biological effects, also act as antioxidants by preventing the oxidation of low density lipoproteins, platelets, aggregation and damage of red blood cells (**Cheyrier, 2005**). Hence, this study was carried out to evaluate water extracts of Chicory and Malva leaves as the antioxidant and anti-inflammatory activities.

MATERIALS AND METHODS

Chemicals and Reagents

λ -Carrageenan, indomethacin and thiobarbituric acid (TBA) were purchased from Sigma-Aldrich Chemical Co. All other chemicals and reagents which used were from the highest analytical grade.

Plant collection and extraction

Chicory (*Cichorium intybus*) and malva (*Malva parviflora* L.) leaves were collected from the farm of Faculty of Agriculture, Cairo University, Egypt. The leaves were identified by the Department of Phytochemistry in the National Organization for Drug Control and Research (NODCAR). Chicory and malva leaves were washed, sliced into thin slices, and dried completely in an air-circulated oven at 40°C. Dried chicory and malva leaves were reduced separately into powder form and sieved through 100-mesh sieve then stored in plastic bags in a refrigerator at 4°C. Two hundred grams of dry powder were subjected to extraction with 2000 ml of water in a screw-capped flask and shaken at room temperature for 24 h according to **Hozayen *et al.* 2016** .The water extract was concentrated under reduced pressure, lyophilized to obtain powders, and stored at 4°C until assay.

Determination of total phenolics content

Phenolic compound contents of chicory and malva were determined calorimetrically using Folin–Ciocalteu reagent (as gallic acid) according to **Singleton *et al.* (1999)**.

Determination of total flavonoids content

Total flavonoids content was determined using aluminium chloride (AlCl_3) according to the method of **Zhishen *et al.* (1999)**. The results were expressed as mg quercetin equivalents/g dry weight of chicory and malva.

HPLC analysis

Phenolic concentrations of chicory and malva leaves were determined by HPLC like the method described by **Goupy *et al.*(1999)**. As follows: 1g of sample was mixed with methanol and centrifuged at 10000 rpm for 10 min ((HERMLE Z206A, Germany) and therefore the supernatant was filtered through a 0.2 μm Millipore after that, it injection into HPLC, using equipped with a variable wave length detector (Agilent, Germany) 1100. Also, the HPLC was equipped with auto sampler, Quaternary pump degasser and column compartment. Analyses were performed on a C18 reverse phase (BDS 5 μm , Labio, Czech Republic) packed stainless-steel column (4 \times 250 mm, i.d.), multi wavelength detector set at 330 nm and 280 nm for detection of flavonoids and phenolic compounds, degasser, column used for fractionation Zorbax OD.4.6 \times 250nm and also the flow rate of mobile phase during run was 1 ml/min. The column temperature was maintained at 35°C. HPLC method started with linear gradient at a flow rate of 1.0 ml / min with mobile phase of water / acetic acid (98: 2 v/v, solvent A) and methanol / acetonitril (50: 50, v/v, solvent B), starting with 5 % B and increasing B to levels of 30% at 25 min, 40% at 35 min, 52% at 40 min, 70% at 50 min, 100% at 55 min. The initial condition was re-established by 5 min wash in both solvents.

***In vitro* antioxidant activity chicory and malva**

The water extracts prepared from chicory and malva were used for the analysis of antioxidant activities by three assays *in vitro*: inhibition of DPPH[•] radical, superoxide anion radical and hydroxyl radical.

1-Hydroxyl radical (OH[•]) scavenging assay

The OH[•] scavenging activity of chicory and malva was determined by the method of Halliwell *et al.* (1987).

2-Superoxide anion scavenging activity assay

The superoxide anion scavenging activity of chicory and malva was determined according to the method of Nishikimi *et al.* (1972).

3- 1,1-diphenyl-2-picrylhydrazyl radical (DPPH[•]) scavenging activity assay

Free radical scavenging activity of chicory and malva was measured using DPPH[•] free radical according to the method of Brand-Williams *et al.* (1995).

4- ABTS⁺ radical scavenging activity assay

The antioxidant activity of the chicory and malva was also measured by ABTS assay (Re *et al.*, 1999).

Experimental Animals

Male white albino rats of Sprague-Dawely strains of 120 -135 g body weights supported from the animal house of NODCAR, Egypt. The animals were kept individually in stainless steel cages at air condition 20-22° C and a relative humidity of about 55%.

Anti-inflammatory activity of chicory and malva

Carrageenan induced paw edema

Thirty male albino rats were divided into five groups each comprised of six rats. Before any treatment, the thickness of the back paw of each animal was determined using an electronic digital caliper, (Germany). Rats were administered orally with indomethacin at a dose 25mg/kg bwt or chicory and malva water extract at a dose 50mg/kg bwt., One hour after these administrations, each rat received in its left back paw a sub planter injection of 1% carrageenan suspension (0.1ml per animal), (Winter et al., 1962). The thickness of the back paw of each rat was measured at 1, 2, 3 and 4 hours after the injection of the carrageenan. The results were presented as the paw thickness (mm).

Biochemical investigation

Two grams of paw tissues were taken, rinsed in ice-cold distilled water and immediately placed in three times their volume of cold 1.15% KCl containing 0.2% Triton X-100 and homogenized. The homogenate was centrifuged at 8000 g for 10 min to obtain the supernatant stored at -20°C (Bicili et al., 2002).

Determination of oxidative stress parameters
Measurement of malondialdehyde (MDA)

MDA content was assayed using the thiobarbituric acid (TBA) test as described by Ohkawa et al., 1979. MDA reacts with TBA to form a colored complex. Absorbance was measured at 532 nm to determine the MDA content. The specific activity is defined as nm/g tissue.

Measurement of reactive oxygen species (ROS) content

ROS was estimated according to **Vrablic *et al.*, 2001**. A modified version of a previously described assay for the intracellular conversion of nitro blue tetrazolium (NBT) to formazan by superoxide anion was used to measure the generation of reactive oxygen species.

Determination of reduced glutathione (GSH)

Levels of GSH were estimated according **Beutler *et al.*, 1963**. Briefly, the deproteinization of homogenate was made by 10% trichloroacetic acid and centrifuged at 3500 rpm for 10 min. 50µL supernatant was mixed with 0.32 mol/L disodium hydrogen phosphate and 0.04% 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) solution. The yellow-colored substance formed by the reaction of GSH and DTNB was measured at 412 nm. The results were expressed as GSH mmol/g tissue.

COX-2 and IL-6 concentrations in Paw Exudates

COX-2 and IL-6 contents were analyzed using a commercially available ELISA kits (BioSource International, Inc., Camarillo, CA, USA) according to the instructions of the manufacturer in supernatant.

Statistical analysis

Statistical analyses were performed with SPSS software and were calculated using one-way ANOVA followed by Post Hoc Duncan. $P < 0.05$ was considered to indicate a statistically significant result.

RESULTS AND DISCUSSION

The flavonoids and phenolic acids are known to possess antioxidant activities due to the presence of hydroxyl groups in their structures and their contribution to defence system against the oxidative damage due to endogenous free radicals is extremely important (Saggu *et al.*, 2014). Polyphenols, are secondary plant metabolites that are found in plants and their products. Many of them have been shown to contain high levels of antioxidant activities (Razali *et al.*, 2008). The total phenolic compounds (as gallic acid) and flavonoids compounds (as quercetin) in dry weight chicory and malva were determined (Table 1).

Table 1. Total polyphenolic and total flavonoid contents of chicory and malva leaves

Constituents	Total phenolic (mg/g GAE)*	Total flavonoid(mg/g quercetin)**
Chicory	3.13 ± 0.10	3.07 ± 0.01
Malva	7.23 ± 0.01	3.35 ± 0.01

*Total phenolic (mg/g): calculated as gallic acid - **Flavonoid (mg/g): calculated as quercetin. Values are mean of three replicates ± SD

The results presented in Table 1 indicated that chicory leaves contained 3.13 mg/g and 3.07 mg/g of total phenolic and flavonoid compounds, respectively. While malva leaves contained 7.23 mg/g and 3.35mg/g of total phenolic and flavonoid compounds, respectively. The high levels of total phenolic and flavonoid content found in chicory and malva are in the range already reported in the literature for chicory Heimler *et al.* 2007 and Lavelli 2007. The results showed that leaves of the chicory and malva are good source of phenolic compounds. Due to high total phenolic and flavonoid content, the leaves have been found to possess comparatively good reducing power.

Polyphenolic compounds of chicory and malva leaves

Qualitative HPLC profile of the chicory and malva leaves was performed using HPLC. Qualitative HPLC analysis of the major peaks of the chicory and malva leaves water extracts was based on the comparison of their retention times with reference standards. The following polyphenols were identified in chicory and malva water extracts: gallic acid, chlorogenic acid, catechin, naringenin, propyl gallate, quercetin, and, cinnamic acid. The major compounds in chicory was ellagic acid and the lower one was propyl gallate (**Table 2**). However, the major compounds in malva was gallic acid and the lower one was cinnamic acid. The results are in agreement with previous findings (**Carazzone *et al.*, 2013, Papetti *et al.*, 2017 and Sahan *et al.*, 2017**).

Thus the plants rich in poly phenols and flavonoids might be a good source of remedial potential against the oxidative stress.

Table 2. HPLC analysis of Polyphenolic and flavonoids of compounds of chicory and malva water extracts.

Compounds	Chicory (µg/g)	Malva (µg/g)
Gallic acid	498.22	539.63
Chlorogenic acid	839.97	152.87
Catechin	200.93	246.95
Coffeic acid	873.17	0.00
Rutin	309.02	0.00
Ellagic acid	1477.07	0.00
Ferulic acid	45.79	0.00
Naringenin	82.20	64.27
Propyl Gallate	20.51	36.53
Querectin	95.65	46.54
Cinnamic acid	25.92	26.87

***In vitro* scavenging activity of chicory and malva leaves**

Figure 1 shows the percentage *in vitro* scavenging effects of the chicory and malva leaves on hydroxyl radical, superoxide anion radical DPPH[•] and ABTS⁺ cation radical. Chicory and malva scavenged these free radicals *in vitro* in a concentration dependent manner. thus from the present study it can be suggested that both the extracts of the chicory and malva may function as scavengers of free radicals by converting reactive free radical species into more stable non-reactive species. High level of antioxidant activity obtained for chicory and malva could be due to its high level of polyphenols.

Usually, the mechanisms of their antioxidant activities are neutralizing lipid free radicals and preventing decomposition of hydroperoxides into free radicals (Javanmardi *et al.*, 2003; Li *et al.*, 2009). These results showed that the studied plants possess higher antioxidant activity and by consequence, they can be considered as good sources of natural products that can be used in the treatment of the different diseases associated to the oxidative stress.

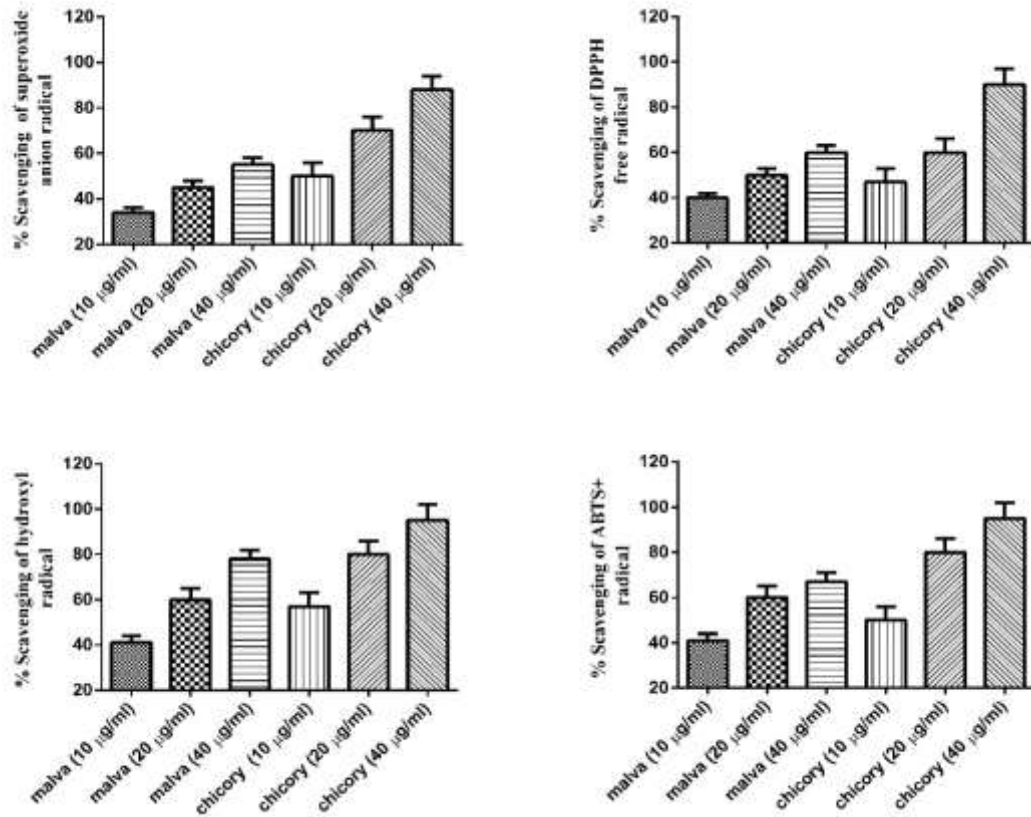


Figure 1. *In vitro* scavenging effects of chicory and malva on hydroxyl radical, DPPH radical, superoxide anion radical and ABTS+ cation radical. The results are expressed as mean±S.D (n=3).

Anti-inflammatory activity of the chicory and malva water extracts on carrageenan-induced paw edema in rats

Inflammation is the body's complicated biological reaction to diseases, irritations or other wounds, and harm to cells. Inflammation plays a significant role in multiple illnesses such as rheumatoid arthritis, asthma, inflammatory intestinal disease, neurodegenerative illnesses, and cancer **Iwalew *et al.* 2007.**

Several pro-inflammatory mediators, including IL-6, IL-12, TNF, COX-2 are published during an inflammatory reaction. We examined the in vitro anti-inflammatory impact of chicory and malva extracts in rats using the carrageenan - induced paw edema model in this research. Injection of carrageenan in rats led to a time-dependent increase in paw thickness (Table 4). Administration of carrageenan increased paw thickness at 1 h and was maximal at 4 h after. However, carrageenan-induced paw edema was significantly reduced ($P<0.05$) in a dose-dependent manner by treatment with chicory and malva water extracts at 3 and 4 h after injection of carrageenan (Table 4).

Table 4. Anti-inflammatory effects of malva and chicory extracts on carrageenan-induced hind paw edema in rats

Groups	Swelling (thickness) (mm)			
	1 hour	2 hours	3 hours	4 hours
Control	11.58±0.003 ^a	11.58±0.005 ^e	11.57±0.003 ^c	11.58±0.003 ^c
Carrageenan	11.46±0.005 ^b	15.91±0.003 ^a	18.50±0.003 ^a	18.59±0.003 ^a
IM (10 mg/kg)	10.17±0.003 ^d	14.52±0.003 ^c	7.27±0.003 ^e	7.10±0.003 ^e
Chicory (50 mg/kg)	10.12±0.003 ^e	14.33±0.003 ^d	7.44±0.003 ^d	8.75±0.003 ^d
Malva (50 mg/kg)	11.16±0.003 ^c	15.01±0.003 ^b	12.49±0.003 ^b	12.20±0.003 ^b

Values are expressed as means±SEM of six rats per group. Values on the same column not sharing the same superscript letters were significantly different ($P<0.05$), IM: indomethacin.

The effect of chicory and malva leaves water extracts on carrageenan-induced paw edema in rats leads to improving paw thickness in a time dependent way following carrageenan injection in rats (Table 4). Intense edema was produced in the carrageenan group, characterized by infiltrates of inflammatory cells as compared to the carrageenan untreated group (normal control) (Table 4).

Chicory and malva leaves water extracts (50 mg/kg) or indomethacin (10 mg/kg) groups treated with carrageenan there were edema decreases as well as decreases in inflammatory cell infiltration. This result showed that chicory and malva extracts are able to reduce the production of inflammatory mediators involved in the development of the acute inflammatory reaction. chicory and malva extracts are rich in flavonoids and polyphenols. These compounds are good inhibitors of serotonin, histamine and leukocyte migration by blocking their adhesion to the vascular wall. **Permender *et al.*, 2009** found that flavonoids have anti-inflammatory activity in both proliferative and exudative of inflammation phases, they inhibit histamine, cytokine, prostaglandins and leukotrienes release. During inflammation, the arachidonic acid cascade is extremely activated, leading in eicosanoid formation, and it is mediated by cyclooxygenase and 5- lipoxygenase enzymes **Heller *et al.* 1998** .It is thought that carrageenan-induced rat paw edema is biphasic. The first stage (1h) includes the release of histamine and serotonin and the second stage (more than 1 hour) is due to the release of drugs similar to prostaglandin. Based on this, a cyclooxygenase inhibition or the exercise of anti-oxidative characteristics may explain the second stage **Salvemini *et al.* 1996** .

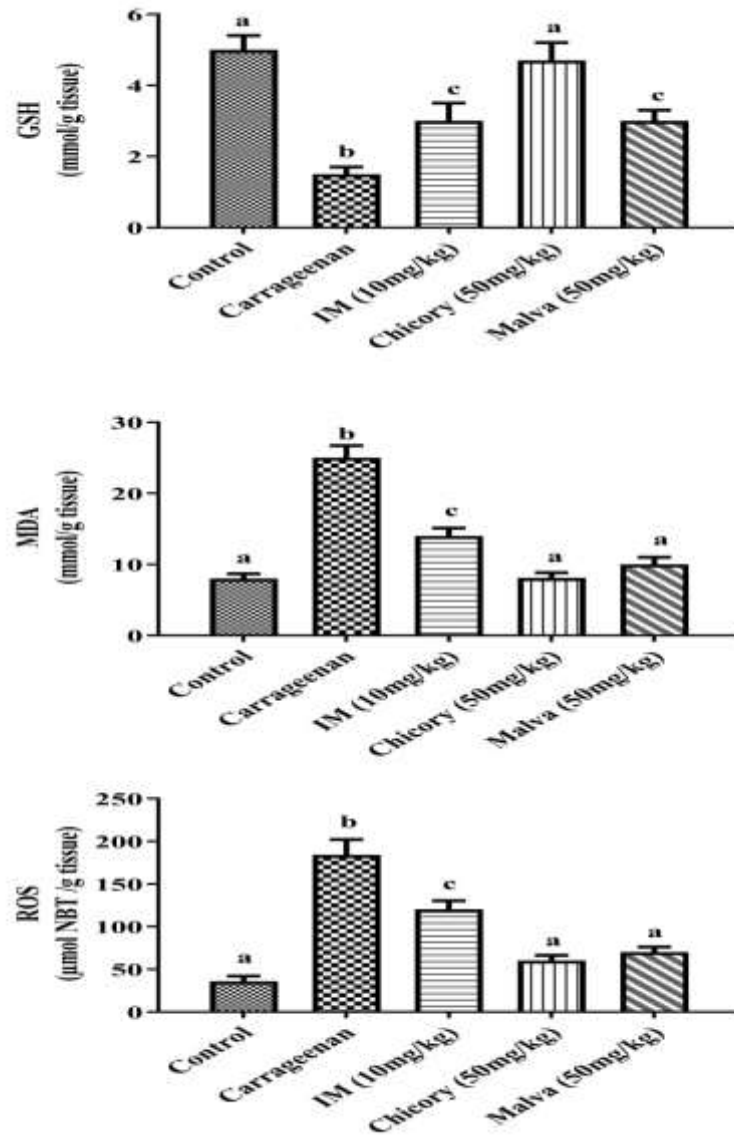


Figure 2. Effect of malva and chicory on carrageenan-induced oxidative stress in rats. Values are expressed as means \pm SEM of six rats per group. Values on the same column not sharing the same superscript letters were significantly different ($P < 0.05$), IM: indomethacin.

Paw tissues were analyzed for biochemical parameters such as ROS, GSH and MDA operations after 5 h of carrageenan paw injection (Fig. 2). After carrageenan therapy, the concentrations of ROS and MDA were considerably increased in the paw tissues. However, in rats treated with carrageenan, GSH content was reduced. Pretreatment of chicory and malva extracts significantly improved ROS, GSH and MDA (Fig. 2). As shown in (Fig. 3) after the injection of carrageenan, the protein expression of IL-6 was considerably improved in the paw tissues, whereas chicory and malva water extracts improved the expression of IL-6 and COX-2. It is well known that the release of IL-1, IL-6, and IL-8 was stimulated by TNF- α . IL-6 improved the COX in turn. In the current research, after carrageenan injection, therapy with chicory and malva water extracts considerably inhibited the paw edema (3-5 h). This stated that chicory and malva water extracts had anti-inflammatory impacts by inhibiting IL-6 and growing antioxidant enzyme GSH with reducing the content of MDA, ROS.

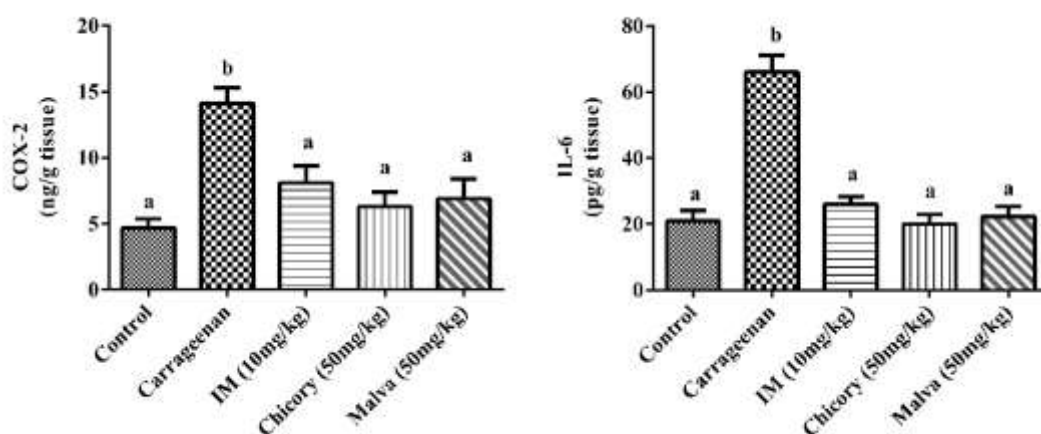


Figure 3. Effect of malva and chicory on COX-2 and IL-6 protein expressions of edema paw in rats. Values are expressed as means \pm SE of six rats per group. Values on the same column not sharing the same superscript letters were significantly different ($P < 0.05$), IM: indomethacin

Our results are consistent with that reported with **Bouriche et al., 2011 and Afolayan et al., 2010** . **Hozayen et al. 2016** reported that chicory represents a promising therapeutic option for the prevention glucocorticoids- induced osteoporosis. Also **Martínez-Hernández et al. (2020)** confirmed that anti-rheumatoid and anti-inflammatory activities of a fraction rich in sterols from *Malva parviflora*.

CONCLUSIONS

The finding of the present study indicates that chicory and malva leaves could be a good source of natural antioxidants and anti-inflammatory and thus improving the human health. Therefore, offers many future applications in field of herbal medicine and nutrition for production of healthy food with well-pronounced healthy effect.

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