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Research Article

ANTIOXIDANT ACTIVITY OF *MAJORANA HORTENSIS* LEAVES SUBJECTED TO OXIDATIVE STRESS IN AN *IN VITRO* SYSTEM

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ABSTRACT
Oxidative stress can arise from an imbalance between the generation and elimination of reactive oxygen species leading to the excess levels, which in turn cause various diseases and cell death. Reactive oxygen species can be eliminated by a number of enzymic and non-enzymic antioxidant defense mechanisms. This was studied in *Majorana hortensis* using *in vitro* model simulating the *in vivo* system. Precision-cut goat liver slices were challenged with a standard oxidant (H_2O_2) both in the presence and in the absence of the different extracts of the leaves. The enzymic and non-enzymic antioxidants were analyzed in the homogenate of the liver slices after incubation. The oxidant treated liver slices showed a decrease in the levels of antioxidants compared to the untreated control. But in the presence of the leaf extracts, the antioxidant status was reverted back to a significant extent. Thus, the results showed that the leaf extracts of the candidate plant can improve the antioxidant status in the goat liver slices exposed *in vitro* to oxidative stress.
KEYWORDS: antioxidants, oxidant, free radicals, reactive oxygen species, oxidative stress.

INTRODUCTION

In the normal metabolic status, the level of free radicals and antioxidants in humans are maintained in balance, which is important for sustaining optimal physiological conditions. Overproduction of free radicals in certain conditions can cause an imbalance, contributing to diseases caused by oxidative damage to biomolocules and altered cellular metabolism[1]. Reactive oxygen species (ROS) can be neutralized by antioxidant defense systems including antioxidant enzymes and antioxidant compounds[2]. Antioxidant supplements or foods rich in medicinal plants are used to help the human body in reducing oxidative damage by free radicals. Currently, research interest has been focussed on the role of antioxidants as well as antioxidant enzymes, in the treatment and prevention of many diseases[3]. Antioxidants may guard against ROS toxicities by the prevention of ROS construction, by disruption of ROS attack, by scavenging reactive metabolites and converting them to less reactive molecules or by enhancing the resistance of sensitive biological target to ROS attack[4]. Candidate plant used in this study is *Majorana hortensis,* commonly called sweet majoram, belonging to the lamiaceae or the mint family. It is a perinneal herb of the Mediterrranean region. Due to its sweet aroma, it has culinary uses. The essential oils present in the leaf enable it to be used for massages. Certain therapeutic remedies are also associated with this leaf; it cures digestive disorders, headaches and fevers. The main objective of the study is to estimate the enzymic and non-enzymic antioxidant potential of the leaves in an *in vitro* model simulating the *in vivo* system, namely, goat liver slices. Organ slices, an *in vitro* model representing the multicellular, structural and functional features of *in vivo* tissue, is a promising model for elucidating mechanisms of drug-induced organ injury and for characterizing species susceptibilities. The liver is the major organ used in organ slice studies[5]. Hence, this study has been carried out using alternative model system, namely goat liver tissues.

MATERIALS AND METHODS

Plant Material: The plant was grown in pots after collecting saplings from Tamil Nadu Agricultural University, Coimbatore and was identified by Botanical Survey of India, Coimbatore as *Majorana hortensis* Moench. (voucher number BSI/SC/5/23/08-09/Tech).

Plant Extract: Methanol and chloroform extracts of the *Majorana hortensis* (*M. hortensis*) leaves were prepared in a 20mg/50 µl concentration of dimethylsulphoxide and used for the assay. An aqueous extract of the fresh leaves was also used to carry out the study.

Goat Liver Slices Preparation: Goat liver was obtained from local slaughter house and maintained on ice throughout the experimental procedure. Precision-cut

goat liver slices weighing 250 mg each was added to tubes containing 1 ml of phosphate buffer saline, pH7.4 respectively and treatment began.

Treatment Groups: The treatment groups set up were negative control without the plant extract and oxidant H_2O_2 and a positive control group which had the liver and oxidant. Three other groups set up were liver slice along with aqueous, methanol and chloroform extracts respectively. Another set of 3 groups were liver with the respective plant extract along with the oxidant. Hence, 8 treatment groups were considered for the experiment.

The concentration of oxidant used was 200 µM. All the respective groups were incubated for 1 hour at 37ºC and then homogenized and centrifuged from which 20 µl of the supernatant was used for the assay.

Evaluation of Enzymic Antioxidants: The assay superoxide dismutase (SOD) was carried out based on method proposed by (Kakkar *et at.,* 1984)[6]. Catalase activity (CAT) was assayed following the method of Luck (1974)[7]. The method proposed by Reddy *et al*. (1995)[8] was adopted for assaying the activity of peroxidase (POD). Glutathione reductase (GR) activity was determined by the method of David and Richard (1983)[9]. Glutathione S-transferase (GST) was assessed by the method of Habig *et al*. (1974)[10].

Evaluation of Non-Enzymic Antioxidant Levels: The nonenzymic antioxidants assessed were vitamin C, E, A and reduced glutathione. Ascorbic acid or viamin C was analysed by the spectrophotometric method described by Roe and Keuther (1943)[11.] Tocopherol or vitamin E was estimated in the plant samples by the Emmerie-Engel reaction as reported by Rosenberg (1992)[12]. Vitamin A was estimated by the method of Bayfield and Cole (1980)[13]. Reduced glutathione was determined by the method of Moron *et al.* (1979)[14].

RESULTS

Enzymic Antioxidant Activity: The activities of the enzymic antioxidants SOD, CAT, POD, GR and GST were analyzed in the liver slices. Effect of *Majorana hortensis* leaves on the antioxidant status in oxidant challenged liver slices of goat is graphically represented**.** H_2O_2 exposure caused a decrease in SOD activity compared to the control group. The co-treatment with the leaf extracts caused a slight elevation in SOD activity. The maximum activity was observed with the methanolic extract treatment (fig 1). A significant decrease in catalase activity was found in H_2O_2-exposed liver slices when compared to the control group (fig 2). Treatment with leaf extracts caused an increase in the catalase activity compared to untreated control. Co-administration of the methanolic extract and the aqueous extract with H_2O_2 caused an increase in the catalase activity. The chloroform extract co-administered group showed a decreased catalase activity compared to untreated control but the activity was higher than the H_2O_2-treated group. The methanolic extract elicited the maximum catalase activity compared to the other two extracts. Similar trend was seen with the activity of peroxidase (fig 3) where a decrease in peroxidase activity by H_2O_2 was counteracted by the administration of aqueous and methanol extracts of the leaves. The activity of chloroform extract revealed a trend much similar to that exhibited for catalase. The liver slices exposed to the methanolic extract of *M. hortensis* leaves showed the maximum activity. The glutathione reductase activities (fig 4) increased in the case of all the three extracts in comparison to the control group. Decreased GR activity was found in the slices exposed to H_2O_2 which was reverted by the administration of all the three extracts of *M. hortensis leaves*, where the methanolic extract was found to be better in minimizing the H_2O_2-induced oxidative stress. The methanolic extract showed significantly higher effect than the aqueous and chloroform extracts. A similar trend was observed in the GST activity as well (fig 5).

Non-Enzymic Antioxidant Levels: The non-enzymic antioxidants, namely vitamins C, E, A and reduced glutathione, were estimated in the oxidant challenged liver slices with or without the leaf extracts of *M. hortensis*. A significant decrease of vitamin C level was found in the H_2O_2 treated group; however, the treatment of the goat liver slices with the leaf extracts reverted the reduction (fig 6). The methanolic and the aqueous extracts caused an increase in the levels of vitamin. Among the three extracts used, the methanolic extract exhibited the maximum protection, followed by the aqueous and chloroform extracts. Similar trend was noted in the case of vitamin E (fig 7). Hydrogen peroxide alone caused a marked decline in the levels of vitamin A, while the trend was effectively reverted by the leaf extract. Among all the extracts used, the liver slices treated with methanolic extract showed more increase in vitamin A level than the groups treated with the aqueous and chloroform extracts (fig 8). The oxidant exposure caused a reduction in the levels of GSH when compared to control. The depleting effect of H_2O_2 treatment was very well counteracted by the administration of the leaf extracts, where the methanolic extract was found to be better than the other two extracts. The activity of chloroform extract revealed a trend much similar to that exhibited by vitamin E (fig. 9).

DISCUSSION

The trans-ferulic acid and gammaoryzanol-treated mice recovered from an ethanol-induced decrease in hepatic

glutathione level by enhancing SOD activity[15]. Gupta *et al*. (2007)[16] have reported that the methanol extract of *Oldenlandia umbellate* exerts a protective effect on hepatic injury by CCl_4 by increasing the activity of catalase. The oral administration of an aqueous extract of *Annona squamosa* leaf combated the streptozotocin-induced oxidative stress by increasing enzymic antioxidants like SOD, CAT and GPx (Kaleem *et al*., 2006)[17]. Prasad *et al*. (2008)[18] reported that lupeol/crude extract of mango pulp treatment resulted in a decrease in ROS levels with restoration in the levels of lipid peroxidation and antioxidant enzymes namely CAT, SOD, GR and GST. GR plays a critical role in maintaining the cell's reducing environment and battling oxidative stress (Berkholz *et al*., 2008)[19]. The results of the work conducted by Ratheesh *et al*. (2010)[20] demonstrated that the alkaloid extract of *Ruta graveolens L.* increased the GSH level in carrageenan-induced acute inflammation and acted as potential antioxidants. According to the work of Kamalakkannan and Prince (2006)[21], oral administration of rutin improved the vitamin E level in streptozotocin-induced diabetic rat tissues, which was attributed to their antioxidant effects.

The outcome of the study clearly demonstrated the antioxidant potential of the *M. hortensis* leaf extracts. The results showed that the leaves possessed high levels of antioxidants, could scavenge neutralize oxidants and free radicals and could improve the antioxidant status of tissue exposed to oxidative stress.

CONCLUSION

The decrease in the use of live animals and the development of alternative models for biomedical research seek to address refinement, reduction and/or replacement (3Rs) of existing animal models. With this as the focus, precision-cut liver slices were used as an *in vitro* system that can simulate the *in vivo* conditions. This system was employed to evaluate the antioxidant potential rendered by the *M. hortensis* leaf extracts against hydrogen peroxide-induced stress *in vitro*. Enzymic and non-enzymic antioxidants were analysed in the goat liver slices subjected to oxidative stress in the presence and the absence of the leaf extracts. The results showed that H_2O_2 exposure caused a significant decrease of all the antioxidants tested, which was effectively reverted by the administration of *M. hortensis* leaf extracts. All the three extracts tested were capable of improving the levels of antioxidants to a significant extent. The methanolic extract was found to be most effective, followed by the other extracts. Thus, the results confirmed that the *M. hortensis* leaf extracts can improve the antioxidant status in oxidatively stressed tissue, which strengthens the antioxidant potential of the plant.

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Fig. 1 Effect of *M. hortensis* leaf on SOD activity

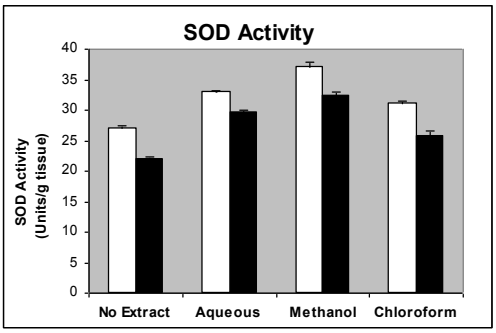


Fig. 2. Effect of *M. hortensis* leaf on SOD activity

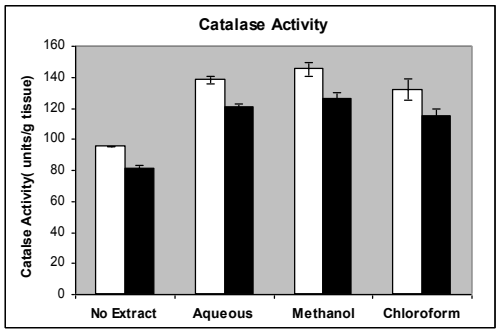


Fig. 3 Effect of *M. hortensis* leaf on Peroxidase activity

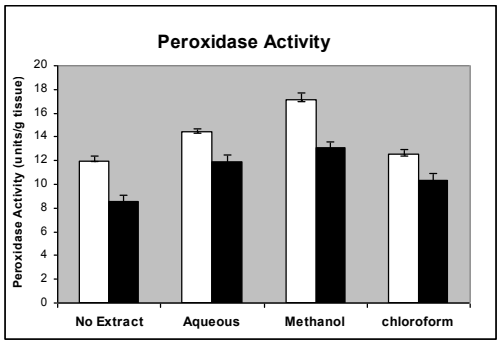


Fig. 4. Effect of *M. hortensis* leaf on GST

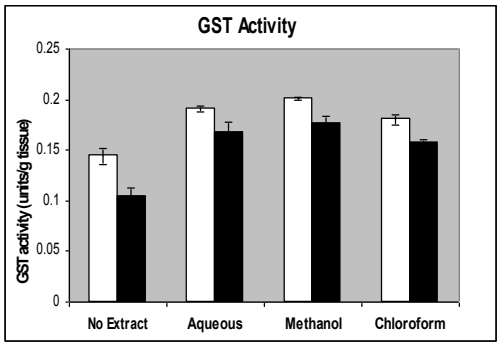


Fig. 5 Effect of *M. hortensis* leaf on GR activity

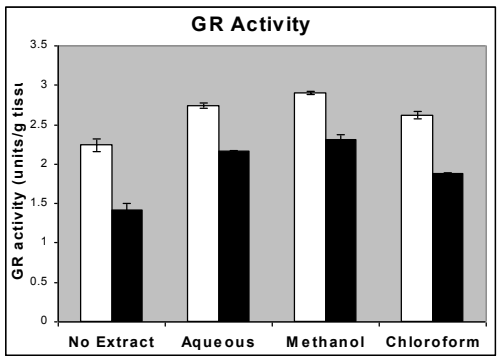


Fig. 6 Effect of *M. hortensis* leaf on Vitamin C activity

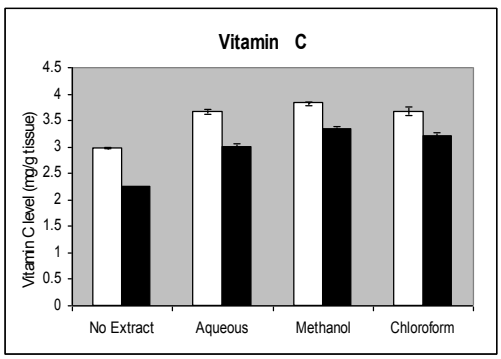


Fig. 7 Effect of *M. hortensis* leaf on vitamin E activity

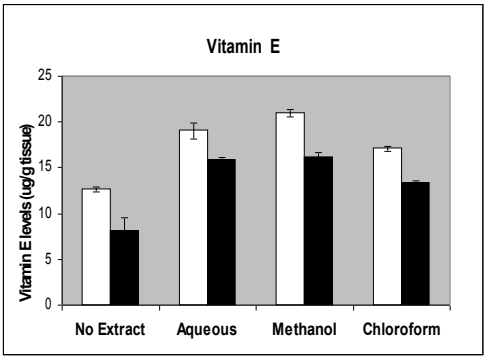


Fig.8. Effect of *M. hortensis* leaf on vitamin A activity

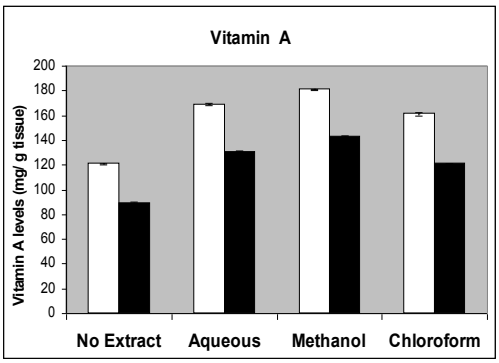
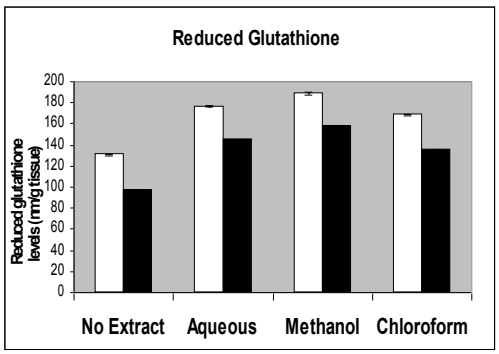
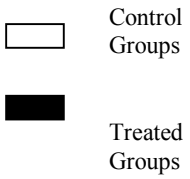


Fig. 9 Effect of *M. hortensis* leaf on Reduced Glutathione activity





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