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Research Article

ANTIOXIDANT EFFECT OF *ARTEMISIA VULGARIS* LEAF EXTRACTS ON OXIDATIVELY STRESSED PRECISION-CUT LIVER SLICES

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ABSTRACT

Oxidative stress results from an excessive production of reactive oxygen species beyond the body's antioxidant capacity. The overproduction of reactive oxygen species can lead to damage to cellular biomolecules, which is implicated in the development of many diseases including cell death. The reactive oxygen species can be eliminated / deactivated by a number of antioxidants which include enzymic and non-enzymic antioxidants. The work was framed to study the antioxidant effects of *Artemisia vulgaris* leaf extracts on oxidatively stressed precision-cut liver slices. Precision-cut liver slices were treated with hydrogen peroxide (standard oxidant) both in the presence and in the absence of three different solvent extracts of the leaves namely aqueous (polar in nature), methanol (partially polar and non-polar) and chloroform (non-polar). The enzymic (superoxide dismutase, catalase, peroxidase, glutathione reductase and glutathione-s-transferase) and non-enzymic (ascorbic acid, tocopherol, vitamin A and reduced glutathione) antioxidants were analyzed in the liver slice homogenate after incubation. Both enzymic and non-enzymic antioxidants were found to be decreased in oxidant-treated liver slices compared to untreated control, whereas the antioxidant activity / level was increased in leaf extract treated slices. This result indicates that the leaf extracts have the ability to improve the antioxidant status in oxidatively stressed liver slices.

Keywords: Antioxidants, Reactive Oxygen Species, Hydrogen Peroxide, Liver slices.

INTRODUCTION

Aerobic organisms are dependent on oxygen, which plays an important role in energy production. Activated oxygen that functions as an oxidant may be represented as a free radical which is generally produced endogenously or also derived exogenously[1]. Reactive Oxygen Species are products of normal cellular metabolism and are well recognized for playing a dual role in living systems. Beneficial effects of ROS occur at low or moderate concentrations and involve physiological roles in several cellular responses. Damage to cellular lipids, DNA and proteins are considered as harmful effects of free radicals[2]. The deleterious effects of oxidative stress can be counteracted by the presence of molecules called antioxidants which are of natural or synthetic sources. But due to the adverse side effects of synthetic antioxidants, search for effective and natural antioxidants has become crucial[3,4]. Several epidemiological studies suggest that plants rich in antioxidants play a protective role in health and against diseases, and their consumption lowered the risk of cancer, heart disease, hypertension and stroke[5]. The candidate plant used in this study is *Artemisia vulgaris,* commonly called as mugwort and belonging to the family of Asteraceae. In traditional medicine, this plant is used for the treatment of diabetes and the extracts of the whole plant are used for epilepsy and in combination of psychoneurosis, depression, irritability, insomnia, anxiety and stress[6]. The main objective of the study was to estimate the enzymic and non-enzymic antioxidant potential of the leaves in an oxidatively stressed *in vivo* simulating *in vitro* system, namely, goat liver slices. Precision-cut liver slices can provide a system where all liver cell-types are present in their natural environment[7], thereby preserving the cell–cell and cell–extracellular matrix interactions. Precision-cut liver slices can contribute to the reduction of animal experiments. Hence, in the present study, precision-cut liver slices were employed as an alternative model system.

MATERIALS AND METHODS

Plant Material

The plant sample was procured from Tamil Nadu, Agricultural University, Coimbatore, India. The plant was grown as pot culture in Avinashilingam University campus. The plant was authenticated by Botanical Survey of India, Coimbatore, India as *Artemisia vulgairs*, L. (Voucher number BSI/SC/5/23/08-09/Tech-1711).

Plant Extracts

The fresh leaves of *Artemisia vulgaris* (5 g) were homogenized in approximately 1.0 ml of solvents namely methanol and chloroform separately. After homogenization, the supernatant was collected and the solvents were evaporated to dry at 60°C and the yields of the extracts were calculated. The residues were reconstituted at 20 mg / 5 µl in dimethylsulfoxide and used for the assay. Apart from the solvent extracts; a fresh aqueous extract was also prepared. A homogenate of the leaves (1 g / 1 ml) was prepared in double distilled water, centrifuged at 2000 rpm for 5 minutes and the supernatant was used as the fresh aqueous extract.

Chemicals

All the chemicals used were of analytical grade.

Preparation of Goat Liver Slices

The goat liver was obtained fresh from a slaughter house and transported to the laboratory on ice. The liver was plunged into ice-cold PBS and maintained at 4ºC till use. The precision-cut slices of 1.0 mm thickness were made. Precision-cut liver slices (0.25 g) were taken in 1.0 ml of PBS in a flat bottomed flask. The liver slices were treated in

the presence or absence of leaf extracts (20 µl) and H_2O_2 (500 µM) and incubated at 37ºC for 1 hour with mild shaking. After incubation, the mixture was homogenized using a Teflon homogenizer. The homogenate was centrifuged at 1500 rpm for two minutes and the supernatant (20 µl) was used for the analysis of various enzymic and non-enzymic antioxidants.

Evaluation of Enzymic Antioxidants

The method described by Kakkar *et al*. (1984)[8] was used for the assay of superoxide dismutase (SOD) activity. The activity of catalase (CAT) was assayed by the method of Luck (1974)[9]. The method proposed by Reddy *et al*. (1995)[10] was adopted for assaying the activity of peroxidase (POX). Glutathione reductase (GR) and glutathione s-transferase activity was determined by the methods of David and Richard (1983)[11] and Habig *et al*. (1974)[12] respectively.

Evaluation of Non-Enzymic Antioxidant Levels

The non-enzymic antioxidants analyzed in the liver homogenate were ascorbic acid, tocopherol, vitamin A and reduced glutathione. The level of ascorbic acid in *Artemisia vulgaris* leaves was quantified spectrophotometrically by the method of Roe and Keuther (1943)[13]. The spectrophotometric method proposed by Rosenberg (1992)[14] was adopted to estimate the level of tocopherol in *Artemisia vulgaris* leaves. Vitamin A was estimated by the method of Bayfield and Cole (1980)[15]. The amount of reduced glutathione present in the leaf sample was estimated by the method proposed by Moron *et al*. (1979)[16].

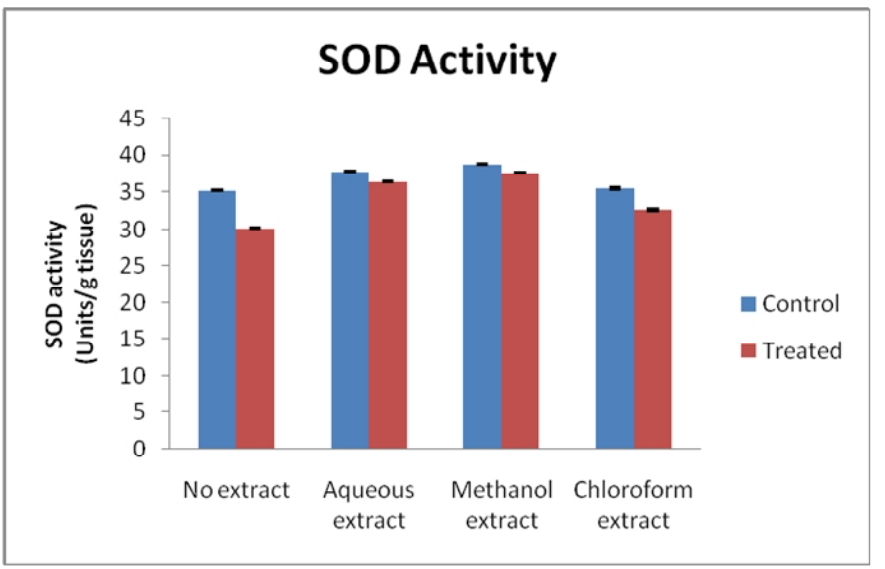

Figure 1: Effect of *Artemisia vulgaris* leaf extracts on superoxide dismutase activity

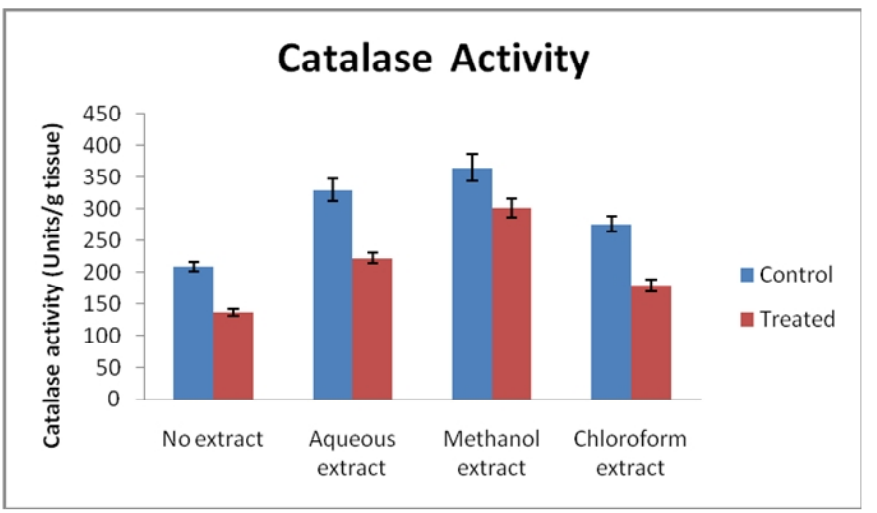

Figure 2: Effect of *Artemisia vulgaris* leaf extracts on catalase activity

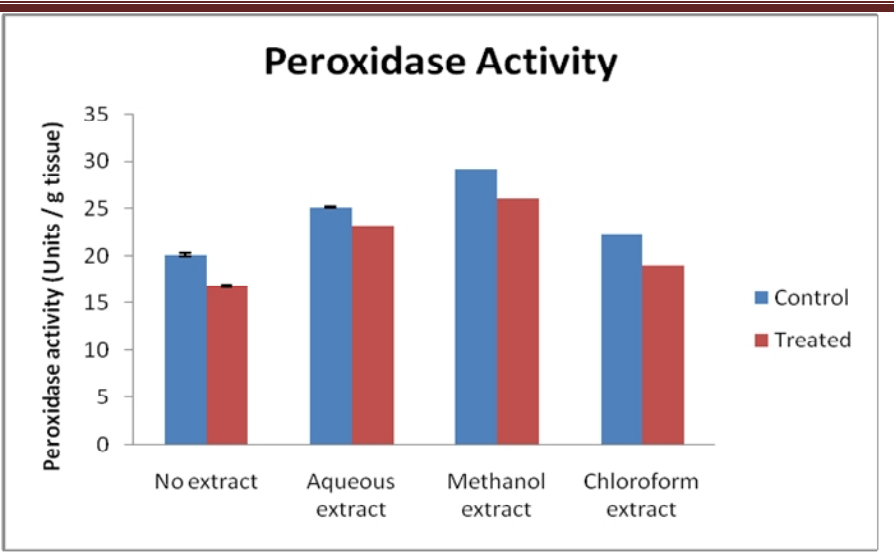

Figure 3: Effect of *Artemisia vulgaris* leaf extracts on peroxidase activity

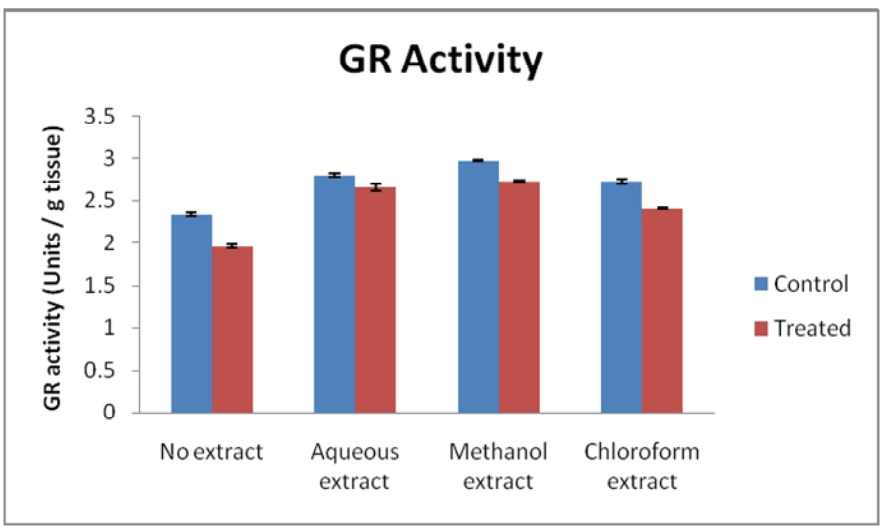

Figure 4: Effect of *Artemisia vulgaris* leaf extracts on glutathione reductase activity

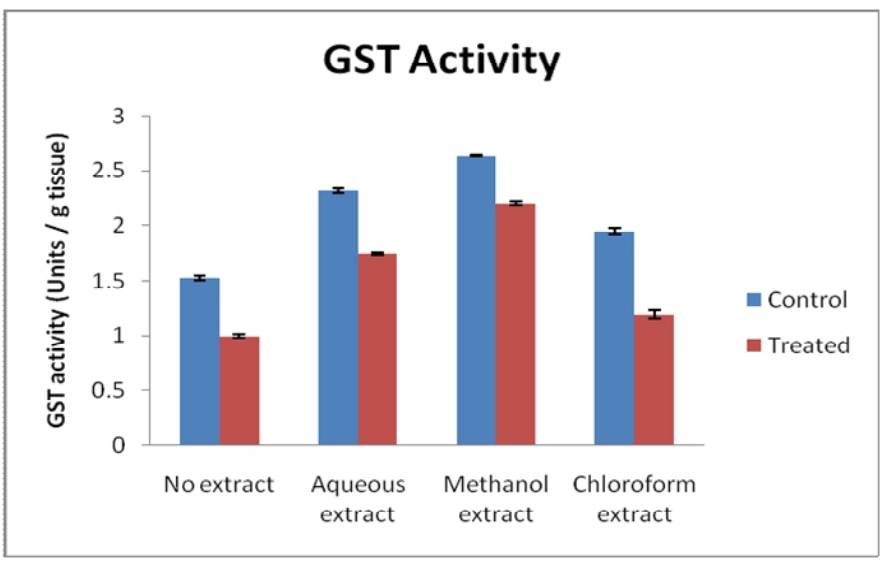

Figure 5: Effect of *Artemisia vulgaris* leaf extracts on glutathione s-transferase activity

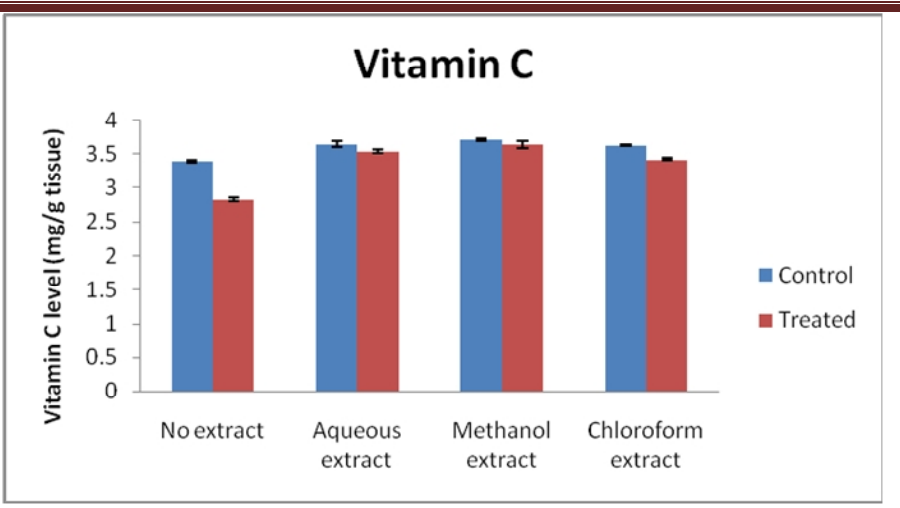

Figure 6: Effect of *Artemisia vulgaris* leaf extracts on vitamin C level

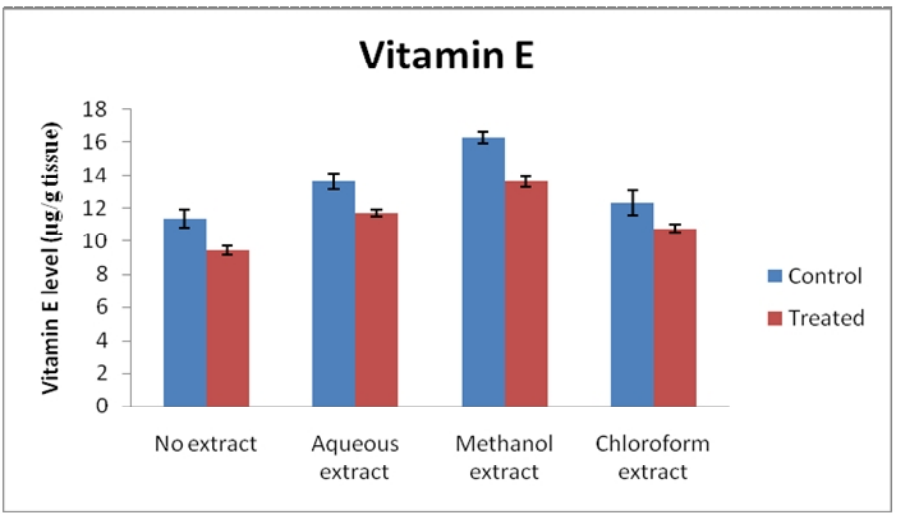

Figure 7: Effect of *Artemisia vulgaris* leaf extracts on vitamin E level

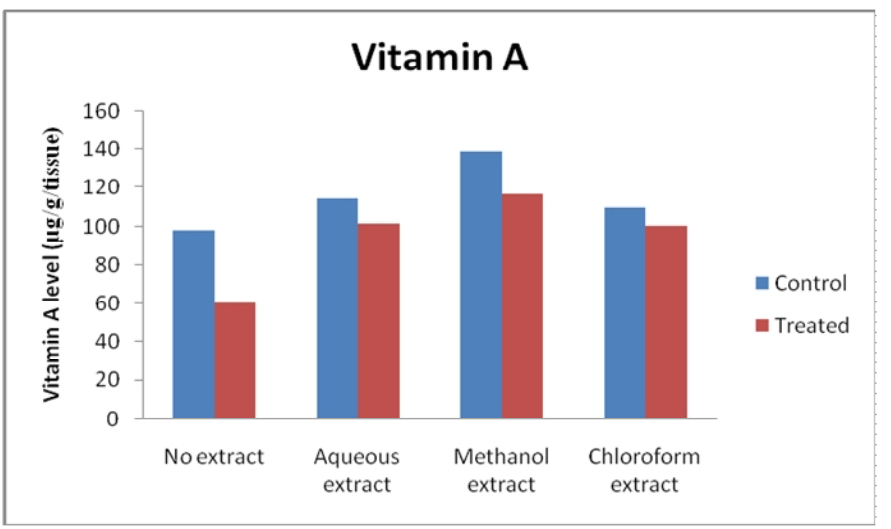

Figure 8: Effect of *Artemisia vulgaris* leaf extracts on vitamin A level

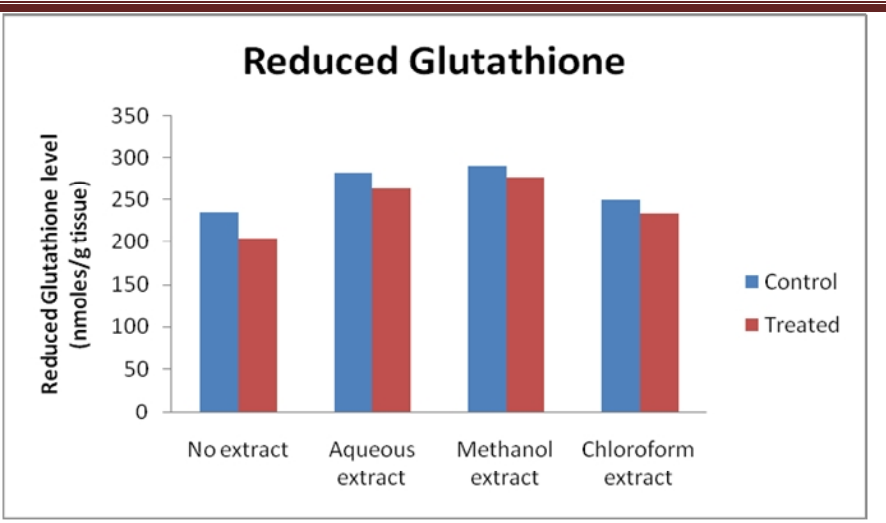

Figure 9: Effect of *Artemisia vulgaris* leaf extracts on reduced glutahione level

RESULTS

Enzymic Antioxidant Activity

The enzymic antioxidant activities namely SOD, CAT, POD, GR and GST were analyzed in the liver slices. Effect of *Artemisia vulgaris* leaf extracts on enzymic antioxidant activities in goat liver slices exposed to H_2O_2 *in vitro* is graphically represented (Figure 1 to 9). H_2O_2 exposure caused a significant decrease in SOD activity compared to the control group. The co-treatment with the leaf extracts caused elevation in SOD activity. The maximum activity was observed with the methanolic extract treatment (Figure 1). A decrease in catalase activity was found in H_2O_2-exposed liver slices when compared to the control group (Figure 2). Co-administration of methanol and aqueous extract with H_2O_2 caused an increase in catalase activity. The chloroform extract co-administered group showed decreased catalase activity compared to untreated control but the activity was higher than the H_2O_2-treated group. The activity of peroxidase decreased upon exposure to H_2O_2 (Figure 3). Treatment with the leaf extracts of *Artemisia vulgaris* caused an increase in the peroxidase activity compared to the control group. The decrease in peroxidase activity by H_2O_2 was counteracted by the administration of aqueous and methanol extracts of *Artemisia vulgaris* leaves. The effect of chloroform extract in peroxidase activity was similar to that of the effect observed for catalase. The glutathione reductase activity increased in all the three extracts in comparison to the control group (Figure 4). Decreased GR activity was found in the slices exposed to H_2O_2. This effect was reverted by the administration of all the three extracts of *Artemisia vulgaris* leaves. H_2O_2 exposure caused a decrease in GST activity (Figure 5). The depletion of GST with the exposure of H_2O_2 was counteracted by the co-administration of leaf extracts. The methanolic extract showed significantly higher effect than the aqueous and chloroform extracts.

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 *A. vulgaris*. Decreased vitamin C level was found in the H_2O_2 treated group (Figure 6). However, the treatment of the goat liver slices with the leaf extracts of *A. vulgaris* reverted the reduction. The methanolic and the aqueous extracts caused an increase in the levels of vitamin C. Among the three extracts used, the methanolic extract exhibited the maximum protection, followed by the aqueous and chloroform extracts. Similar trend was observed in vitamin E level (Figure 7). Hydrogen peroxide alone caused a marked decline in the levels of vitamin A, while the trend was effectively reverted by the *Artemisia vulgaris* leaf extracts. 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 (Figure 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 (Figure 9).

DISCUSSION

Many studies have shown that the administration of herbal extracts can improve the antioxidant status of tissues, both *in vivo* and *in vitro*. Liu *et al* (2009)[17] reported that a diet enriched with protandium, a combination of five phytochemicals from medicinal plants, improved SOD activities and suppressed tumor promoter-induced oxidative stress in mice. Gupta *et al*. (2007)[18] have reported that the methanol extract of *Oldenlandia umbellate* exerts a protective effect on CCl_4 - induced hepatic injury by increasing the activity of catalase in rats. Mahesh *et al*. (2007)[19] demonstrated that the administration of an aqueous extract of *Terminalia chebula* showed marked increase in GPx activity in aged rat brain, which was suggested to be due to the protection of sulphydryl groups in glutathione from oxidative damage. The activities of glutathione reductase and glutathione S-transferase were decreased in CCl_4 and N-nitrosodiethylamine injured rat liver, which were significantly preserved by the synergistic effect of silymarin and garlic[20]. Visavadiya and Narasimhacharya (2009)[21] reported that *Asparagus racemosus* root powder improved the status of antioxidants namely ascorbic acid, SOD and CAT in hypercholesterolemic rats. Soussi *et al*. (2006)[22] demonstrated that the pre treatment with green tea (*Camellia sinensis*) significantly improved the levels of vitamins E and A in the liver and kidney of rats with ammonium metavanadate-induced toxicity. An aqueous extract of *Ocimum sanctum* increased the level of GSH in alcohol treated rats (Shetty *et al*., 2008)[23]. The outcome of the present study showed that the leaves of *A. vulgaris* possessed high

levels of antioxidants which could effectively protect the stress induced by oxidants in goat liver slices. Sreelatha and Padma (2010)[24] reported that CCl_4 treatment significantly decreased the activities of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione S-transferase. Treatment with *Moringa oleifera* extract increased the activities of antioxidant enzymes and glutathione content significantly. The activity of enzymic antioxidants and the levels of non enzymic antioxidants which decreased initially by H_2O_2 treated goat liver slices was found to be increased on treatment with methanolic extract of both the leaves and rhizomes of *Curcuma amada* (2012)[25]. The study carried out by Radha and Padma (2011)[26] revealed that the methanol and chloroform extracts of *Majorana hortensis* leaves can improve the antioxidants (enzymic and non-enzymic) status of liver slices exposed to oxidative stress.

CONCLUSION

In general, groups promoting the 3Rs (refinement, reduction and/or replacement) of animal welfare for biomedical research have overlooked the immediate welfare gains that may be possible using various *in vitro* systems as alternative models. With this as the focus, precision-cut liver slices were used as an *in vitro* system that simulate the *in vivo* conditions prevailing in experimental animals and perhaps, the human system. The precision-cut liver slices provide a system in which the cells are present in their natural environment. This system was employed to evaluate the antioxidant potential rendered by the *Artemisia vulgaris* leaf extracts against hydrogen peroxide-induced stress *in vitro*. All the three extracts tested were capable of improving the levels of antioxidants studied to a significant extent. The methanolic extract was found to be most effective, followed by the aqueous and chloroform extracts. Thus, the results confirmed that the *Artemisia vulgaris* leaf extracts can improve the antioxidant status in oxidatively stressed tissue, which strengthens the antioxidant potential of the plant.

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