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MODULATORY ROLE OF *ARUMUGA CHENDOORAM* ON PLASMA ANTIOXIDANT IN EXPERIMENTAL HYPOTHYROID RATS

R. Albert Santhanaraj Deepak[#] and V. Elango*

[#]Research Scholar, Department of Siddha Medicine, Tamil University, Thanjavur District, Tamil Nadu, S. India

*Department of Siddha Medicine, Tamil University, Thanjavur District, Tamil Nadu, S. India.

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For Correspondence:

V. Elango

Department of Siddha Medicine, Tamil University, Thanjavur District, Tamil Nadu, S. India

ABSTRACT

The methimazole treated rats were administered *Arumuga chendooram*. Malondialdehyde (MDA) was marked increased and also a distinct diminution in glutathione (GSH) content in the plasma of methimazole treated rats. In *Arumuga chendooram* treated rats these biochemical parameters attained an almost normal level. The decreased activity of plasma antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-s-transferase and non-enzymatic antioxidant, such as glutathione (GSH), vitamin C and vitamin E in methimazole treated rats, and its retrieval towards near normalcy in *Arumuga chendooram* administered rats. The efficacy of *Arumuga chendooram* in combating methimazole treated induced oxidative stress. From these results, it was suggested that oxidative stress had been nullified due to the effect of *Arumuga chendooram*.

INTRODUCTION

Antioxidants can delay or inhibit the oxidation of lipids or other biomolecules by inhibiting the initiation or propagation of oxidative chain reactions (Sen*et al*., 2010). Free radicals mediate some of the reactions of oxidants. They are therefore implicated as agents of oxidative stress. Although reactive oxygen species (ROS) and reactive nitrogen species (RNS) play important roles in many biological processes and are involved in host defense (Eze, 2006). The most common Reactive oxygen species (ROS) include superoxide ($O_2^{\bullet-}$) anion, hydrogen peroxide (H_2O_2), peroxyl ($ROO^{\bullet}$) radicals, and the very reactive hydroxyl ($OH^{\bullet}$) radicals. The nitrogen-derived free radicals are nitric oxide ($NO^{\bullet}$) and peroxy nitrite anion ($ONOO^{\bullet}$). Overproduction of these species may contribute to the immunopathological phenomena related to oxidative stress. ROS are also implicated in the pathogenesis of a vast variety of conditions including inflammatory diseases, diabetes mellitus, malaria, neurodegenerative diseases, HIV/AIDS (Velavan, 2011) and aging (Rattan, 2006). Antioxidants have been reported to prevent oxidative damage by free radical and ROS, and may prevent the occurrence of disease, cancer and aging. It can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals, and also by acting as oxygen scavengers (Buyukokuroglu*et al*., 2001; Shahidi and Wanasundara, 1992). In searching for novel natural antioxidants, some herbal formulations have been extensively studied in the past few years for their antioxidant and radical scavenging components. Keeping this in view, the present study was to monitoring the effect of *Arumuga chendooram* on antioxidant status in experimental hypothyroid rats.

MATERIALS AND METHODS

Animals

Male albino rats of Wistar strain approximately weighing 180-190g were used in this study. They were healthy animals purchased from the Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2º C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided *adlibitum*. They were acclimatized to the environment for one week prior to experimental use. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Chemicals

Nitrobluetetrazolium (NBT), ethylenediaminetetra acetic acid (EDTA), Trichloro acetic acid (TCA), Thiobarbituric acid (TBA), 5,5'-dithio-bis (2-nitrobenzoic acid), glutathione (reduced), glutathione (oxidized) and Nicotinamide adenine dinucleotide phosphate ($NADP^{+}$/NADPH) were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used were of analytical grade and were obtained from Glaxo Laboratories, Mumbai, India, and Sisco Research Laboratories, Mumbai, India.

Preparation of *Arumuga chendooram*

The Siddha medicine *Arumuga chendooram* was prepared at its different stages of preparation in departmental laboratory with the help of a traditional siddha medical practioners as per the IMCOPS method.

In the first stage of the preparation of *Arumuga chendooram*. Five parts of purified mercury (Suththiseitharasam), nine parts of purified sulphur (Suththiseithakanthakam), seven parts of purified lode stone (Suththiseithakantham), twelve parts of purified iron filing (Sutht.hiseithaayapodi), four parts of rock salt (Induppu) and eight parts of desiccated borax (Poriththavenkaram) were ground with sufficient quantity of aloe juice (Kumaricharu for five days continuously. This was then made into small cakes and dried. It was then sealed in discs and burnt for 24 hours. If the colour of the chendooram does not appear as dark purple the grinding and burning are usually repeated equal to pH and then attractive particle interations predominate which may influence the drug delivery.

Experimental design

Body weights of the animals were recorded and they were divided into **4** groups of 6 animals each as follows. First group was normal rats fed with standard diet and served as a control which received saline. Second group was negative control administered Methimazole (40mg/kg) induced experimental hypothyroidism for 40 consecutive days Third group was treatment group treated with Methimazole (40mg/kg) along with *Arumuga chendooram* (10mg/kg) for 40 days. Fourth group was positive control treated with Methimazole (40mg/kg) along with standard throxine sodium (20µg/kg) for 40 days.

Collection of samples

On completion of the experimental period, animals were anaesthetized with thiopentone sodium (50mg/kg). The blood was collected with or without EDTA as anticoagulant. Plasma was separated for the estimation of various biochemical parameters.

Biochemical Estimation

Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust (1978). Reduced glutathione was estimated by method of Moron *et al* (1979). The level of ascorbic acid was estimated by the method of Omaye*et al* (1979). α-tocopherol was estimated by the method of Baker *et al* (1980). Copper-zinc superoxide dismutase activity was determined by the procedure of Kakkar*et al.* (1984) in plasma. The activity of catalase was assayed by the method of Beers and Sizer (1952).The activity of glutathione peroxidase was assayed by the method of Rotruck*et al* (1973).

RESULTS AND DISCUSSION

The methimazole treated rats were administered *Arumuga chendooram*. Malondialdehyde (MDA) was marked increased and also a distinct diminution in glutathione (GSH) content in the plasma of methimazole treated treated rats. In *Arumuga chendooram* treated rats these biochemical parameters attained an almost normal level. The decreased activity of plasma antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-s-transferase and non enzymatic antioxidant, such as glutathione (GSH), vitamin C and vitamin E in methimazole treated treated rats, and its retrieval towards near normalcy in *Arumuga chendooram* administered rats (Table 1 and fig. 1). The efficacy of *Arumuga chendooram* in combating methimazole treated induced oxidative stress. From these results, it was suggested that oxidative stress had been nullified due to the effect of *Arumuga chendooram*.

Table 1 Effect of *Arumuga chendooram* on plasma antioxidant defence in experimental rats

	Group I	Group II	Group III	Group IV
MDA	11.35±0.79 [a]	19.65±1.37 [b]	12.65±0.88 [a]	12.06±0.84 [a]
GSH	8.56±0.59 [a]	5.30±0.37 [b]	7.95±0.55 [a]	8.06±0.56 [a]
SOD	4.18±0.29 [a]	2.53±0.17 [b]	4..04±0.28 [a]	3.98±0.27 [a]
Cat	8.47±0.59 [a]	5.60±0.39 [b]	7.86±0.55 [a]	8.02±0.56 [a]
GPX	7.16±0.50 [a]	5.12±0.35 [b]	6.92±0.48 [a]	7.03±0.49 [a]
Vit C	3.50±0.24 [a]	1.85±0.12 [b]	3.32±0.23 [a]	3.21±0.22 [a]
Vit E	2.45±0.17[a]	1.10±0.07[b]	2.27±0.15 [a]	2.34±0.16 [a]

Each value is expressed as mean ± SD for six rats in each group.

[a]As compared with group I, [b]As compared with group II, III and IV. [*]$p<0.05$.

MDA: nmol of MDA formed/L; SOD, CAT, GPx U/ml; GSH, Vit C, Vit E mg/dl.

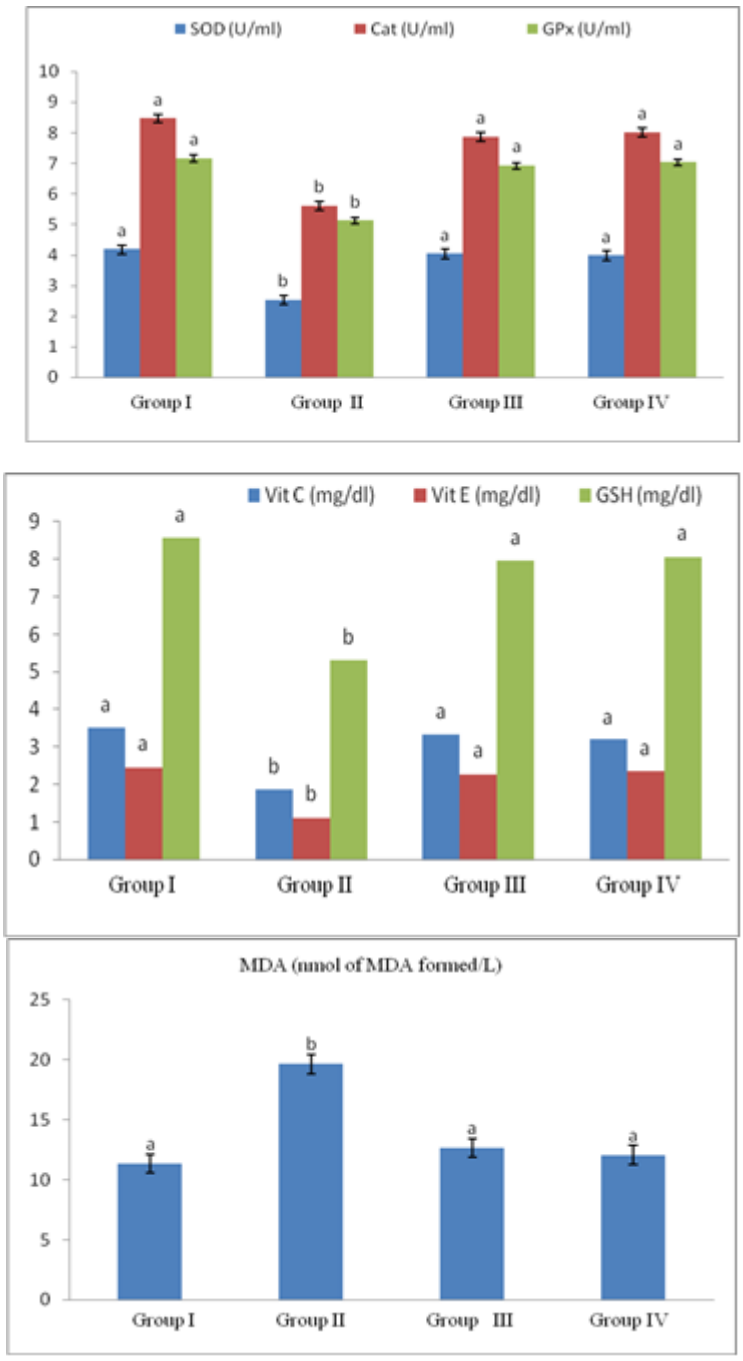

Figure 1 Effect of *Arumuga chendooram* on plasma antioxidant defence in experimental rats

Each value is expressed as mean ± SD for six rats in each group.

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MDA: nmol of MDA formed/L; SOD, CAT, GPx U/ml; GSH, Vit C, Vit E mg/dl.

The thyroid gland is the most important endocrine gland for metabolic regulation (Cunningham, 2002). The thyroid hormones, thyroxin (T4) and triiodothyronine (T3) are necessary for the growth, metabolism and functioning of virtually every cell in the body (Sojka, 1995). Hypothyroidism is a common thyroid disorder which may give rise to goiter, that is, an enlarged

thyroid. Hypothyroidism is commonly due to deficient iodine intake or secondarily due to intake of goitrogens (Radostits*et al*., 2000). Recent studies have shown an increased production of reactive oxygen species in hypothyroidism (Sarandol*et al*., 2005; Erdamar*et al*., 2008).

Redox metabolism corresponds to a complex interacting network involving the generation of reactive oxygen species and enzymatic and non-enzymatic cellular antioxidant defenses. Any small and transient disturbance of this balance induces redox signaling, which can act on several transduction pathways or enzyme and transcription factor activities. In contrast, when antioxidant defenses are chronically overwhelmed, the result is an oxidative stress in which free radicals may exert their deleterious effects (Droge, 2002). Oxidative stress has been implicated in the pathogenesis of several metabolic diseases as well as in the comorbidity of diabetes mellitus, obesity and atherosclerosis. In such studies, redox metabolism was evaluated by the pattern of various parameters such as manganese dependent superoxide dismutase, glutathione peroxidase or catalase activities, and glutathione or α-tocopherol content in blood and liver (Touyz and Schiffrin, 2004; Faure *et al.,* 2004).

Malondialdehyde (MDA) is the major aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acid. MDA, a secondary product of lipid peroxidation is used as an indicator of tissue damage by series of chain reactions (Ray and Husain, 2002). The study of lipid peroxidation is attracting much attention in recent years due to its role in diseases process membrane lipids are particularly susceptible to lipid peroxidation due to the presence of polyunsaturated fatty acids. It has been implicated in the pathogenesis of a number of diseases and clinical conditions. These include atherosclerosis, cancer etc., Experimental and clinical evidence suggests that aldehyde products of lipid peroxidation can also act as bioactive molecule in physiological and pathological conditions. It is now generally accepted that lipid peroxidation and its product play an important role in liver, kidney, heart and brain toxicity (Lakshmi *et al*., 2005). MDA is one of the indicators of oxidative stress. In this context a marked increase in the concentration of MDA was observed in methimazole treated rats when compared to control rats. Administration of *Arumuga chendooram* to methimazole treated rats significantly decreased the levels of MDA in Group III rats.

Plasma is endowed with innate antioxidant defense mechanisms, including the enzymes catalase,superoxide dismutase, glutathione peroxidase and glutathione-S-transferase. CAT is responsible for the detoxification of significant amounts of H_2O_2. SOD catalyses the removal of superoxide radical (O_2•-), which would otherwise damage the membrane and biological

structures. GST is actually composed of a group of isoenzymes capable of detoxifying various exogenous and endogenous substances by conjugation with glutathione. A reduction in the activity of these enzymes is associated with the accumulation of highly reactive free radicals, leading to deleterious effects such as loss of integrity and function of cell membranes. A decrease has been observed in the activity of CAT, SOD and GPx enzymes in plasma (Uma Bhandari*et al.*, 2013) of rats that had received HFD. Noeman*et al*. (2011) suggested that the reduced activities of these enzymes might reflect a feed-back inhibition or oxidative inactivation of protein caused by excess generation of ROS. So also, in the present study, significantly lower activities of these enzymes were noted in rats, when compared to the levels in normal rats. Administration of *Arumuga chendooram* to methimazole treated rats markedly elevated the levels of these antioxidant enzymes so that they approximated the levels seen in normal rats. Increased activity of SOD enzyme in *Arumuga chendooram*s treated rats divulges that *Arumuga chendooram* may act as a potent scavenger of superoxide radicals and metal chelator reported in our study. The *Arumuga chendooram* treated group showing increase in CAT activity may be explained by the free radical scavenging action of components present in the *Arumuga chendooram*. Similar elevation of these enzyme levels by supplementation of antioxidant source as *Embeliaribes* extract has already been reported (Uma Bhandari*et al.*, 2013).

Glutathione is a ubiquitous thiol containing tripeptide, which plays a central role in cell biology. It is implicated in the cellular defence against xenobiotics and naturally occurring deleterious compounds, such as free radicals and hydro peroxides. Glutathione status is a highly sensitive indicator of cell functionality and viability. GSH depletion is linked to a number of diseases states including cancer, neurodegenarative diseases, kidney and cardiovascular diseases. Thus the GSH concentrations in plasma are important (Pastore*et al*., 2003). In the present study, a marked decrease in the concentration of GSH was observed in methimazole treated rats when compared to control rats. Administration of *Arumuga chendooram* methimazole treated rats significantly increased in the levels of GSH in Group III rats.

Living organisms have developed complex antioxidant systems to counteract reactive oxygen species. These antioxidant systems include enzymes such as superoxide dismutase, catalase and glutathione peroxidase; macromolecules such as albumin, ceruloplasmin and ferritin and a variety of small molecules, including ascorbic acid, alpha-tocopherol, reduced glutathione, methionine, uric acid and bilirubin (Goraca and Skibska, 2005).

An antioxidant has been defined as "any substance that present at low concentrations compared with those of an oxidizable substrate significantly delays or prevents oxidation of that substrate". When ROS /RNS are generated in view, their actions are opposed by intricate and coordinated antioxidant lines of defense systems. This includes enzymatic and non – enzymatic antioxidant that keeps in check ROS / RNS level and repair oxidative cellular damage (Halliwell and Gutteridge, 1999). Circulatory non-enzymatic antioxidant such as vitamin E and vitamin C are free radical scavengers. Their synergestic action in scavenging oxygen-derived free radicals is well documented (Wojacki*et al*., 1995). Vitamin E reacts with lipid peroxy radicals acting as a chain terminator of lipid peroxidation while vitamin C helps to maintain the level of vitamin E at optimum concentrations. Serum levels of vitamin E and vitamin E in the present study were significantly reduced in methimazole treated rats as compared with control. This reduced content of vitamin C and vitamin E indicates that utilization for scavenging of free radicals which are produced in methimazole detoxification in liver. Administration of *Arumuga chendooram* to methimazole treated rats significantly increased in the levels of vitamin C and vitamin E in Group III rats.

The results of the present study indicate that the protective role of *Arumuga chendooram* on oxidative stress induced by methimazole treated rats may be related to a counteraction of free radicals by its antioxidant activity of *Arumuga chendooram*, to strengthen endogenous antioxidant defense by its ability to increase the non enzymatic antioxidants like GSH, vitamin C and vitamin E decreased content of lipid peroxide which is used as a marker for oxidative stress. This protective activity of *Arumuga chendooram* is mainly attributed to the presence of enriched therapeutic phytochemical constituents, which act synergistically to detoxify the free radicals produced by methimazole and thereby decrease oxidative stress.

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