Reactive oxygen species (ROS) are an important group of molecules.

INTRODUCTION

Amongst the spectrum of sources for free radical mediated oxidative stress, changing lifestyle pattern nowadays may be a good booster of oxidative stress induced systemic disorders [5]. Eye attracting coloured foods consumption in our country like roadside cheap and hotel made biriyani, mango and milk shakes, cheese and sweets, ladoos has become a common fashion amongst inhabitants belonging to low and medium socio-economic status in metropolitan cities. Such foods are generally adulterated with metanil yellow (as observed from our laboratory studies) [6]. Consumption of such food adulterant may alter a metabolic pattern that may account for oxidative stress induced systemic disorders [5]. Eye attracting coloured foods consumption in our country like roadside cheap and hotel made biriyani, mango and milk shakes, cheese and sweets, ladoos has become a common fashion amongst inhabitants belonging to low and medium socio-economic status in metropolitan cities. Such foods are generally adulterated with metanil yellow (as observed from our laboratory studies) [6]. Consumption of such food adulterant may alter a metabolic pattern that may account for oxidative stress induced systemic disorders [5].

Antioxidant supplementation is widely followed the regime to combat cellular oxidative insult. Amongst these groups, synthetic antioxidant supplantation has been criticized my many and shifting of therapy towards the use of indigenous plant-based chemicals (including polyphenols, flavonoids, phenolic acids, tannins etc.) is recently gaining recognition [8]. One such plant widely used by Indians in diet, popular for its delicious taste is Coriandrum sativum. Apart from raw consumption of its leaves, seeds of this plant are also used as common Indian spice. Essential oils extracted from leaves, seeds, fruits etc. is used as folk medicine for curing bacterial, fungal diseases and antioxidant therapy [9]. Coriandrum leaves have been reported to have anti-diabetic, anti-atherosclorotic and several other medicinal uses [10]. Studies showed that Coriandrum leaves extract has anticancer activity [11]. Studies also showed that reversal of memory deficit in mice model is possible using Coriandrum leaves extract [12]. Coriandrum sativum leaves extract has been found to have radical scavenging potential and it has the

ABSTRACT

Objective: The primary aim of the study was to find out a suitable effective dose of metanil yellow that can cause alterations of lipid peroxidation and catalase activity in the heart in vitro and to determine the effectiveness of graded doses of Coriandrum sativum as an antioxidant in preventing lipid peroxidative damage to cardiac tissue and back up catalase activity.

Methods: Metanil yellow at different doses (10, 25, 35, 50 µl in phosphate buffer) were applied on isolated hircine heart homogenate (10%) in in vitro followed by estimation of lipid peroxidation and catalase activity after completion of the incubation period. Another set of the experiment included incubation of the heart homogenates with graded dilutions of ethanolic leaf extracts of Coriandrum sativum (CsEtH) at concentrations of 10, 20, 30, 40 µl in phosphate buffer medium followed by quantification of the same biochemical parameters as above. This was followed by co-administration of the selected effective dose of metanil yellow (35 µl) and ethanolic leaf extract of Coriandrum sativum (30 µl) on heart homogenate to track the variation in lipid peroxidation and catalase activity in heart homogenate in vitro.

Results: Metanil yellow significantly lowered catalase activity in isolated cardiac tissue that was reflected in elevated lipid peroxidation assuming free radical induced oxidative injury to cardiac tissue [p<0.01]. However ethanolic leaf extract of Coriandrum sativum buffered such changes (p<0.001) significantly, presuming a protective action against oxidative cardiac damage.

Conclusion: It may be concluded that ethanolic leaf extract of Coriandrum sativum is a promising cardioprotective remedy not only in mitigating free radical mediated lipid peroxidative damage to cardiac tissue but also in reinstating normal catalase activity that may be used an unique herbal based remedy for such cardiac ailments in future.

Keywords: Cardioprotection, Coriandrum sativum, Metanil yellow, Methanolic leaf extract, Myocardial infarction

INTRODUCTION

Reactive oxygen species (ROS) are an important group of molecules that are generated usually by oxygen slippage either during mitochondrial electron transport or extra-mitochondrial electron transport systems [1]. An imbalance between the generation of such free radicals and scavenging activity of these free radical by cellular antioxidant enzyme cascade may be a cause for generation of oxidative stress. Oxidative stress may be the etiology of a multitude of ailments ranging from neurological, haematological, immunological, cardiovascular, and genetic to developmental disorders [2].

Mammalian heart is highly aerobic and oxygen demanding organ. In addition, cardiac tissue is normally dynamic with highly metabolic demand and mitochondrial rich myocardial work out may be a source of oxygen slippage turning out as oxidative injury to the heart. ROS generation in the heart may affect normal myocardial contractility induce maladaptive myocardial remodelling and subsequently, heart failure may be an outcome [3].

Cellular antioxidant mechanisms work in cascade in the heart to combat the stress mediated cardiac injury. Formerly superoxide anion generation are prevented by vitamin E, formation of hydrogen peroxides from superoxide radicals are again checked by sulphur containing amino acids like cystine and methionine, and later generation of hydroxyl radicals are counteracted by coordinated actions by superoxide dismutase and catalase which otherwise may interact with polyphenolic lipids to form lipid hydroxides, a product of lipid peroxidation. Although SOD and glutathione peroxidase are adequate in cardiac tissue to combat oxidative damage, catalase is very crucial for H2O2 detoxification in cardiac myocytes [4].

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Original Article

BENEFICIAL EFFECTS OF ETHANOLIC LEAF EXTRACT OF CORIANDRUM SATIVUM ON METANIL YELLOW INDUCED ALTERATION INACTIVITY OF CATALASE AND LEVEL OF LIPID PEROXIDATION IN HERCINE CARDIAC TISSUE IN VITRO
ability to protect DNA damage [13]. *Coriandrum sativum* leaves extract has been reported to scavenge DPPH and has antibacterial [14]. Our earlier studies reveal that methanolic extract of the leaves of *Coriandrum sativum* has hepatoprotective effect in vitro [5]. However cardioprotective effects of ethanolic leaf extract of this plant is yet to be scrupulously determined.

One of the principal aims of this study was to explore the possible effects of metanil yellow on H$_2$O$_2$ metabolism by monitoring catalase activity which may partially anticipate the mechanism of activation of lipid peroxidation in cardiac tissue in vitro. The secondary aspect of this study was to find out an effective dose of this drug in producing such oxidative insult.

**MATERIALS AND METHODS**

**Collection of Coriandrum sativum plant**

The shoots of *Coriandrum sativum* were collected from the local market. Plant along with its seeds was collected from Chinsurah Hooghly, West Bengal, herbarium was prepared and the specimen was identified by a concerned expert from the Botanical Survey of India, Central National Herbarium as *Coriandrum sativum* [Specimen voucher number is HMC/SG/PHYSIO-02].

**Processing of the leaves**

Fresh leaves were isolated from the plant, washed and dried on filter paper at normal room temperature. The dried leaves were weighed. The leaves were then placed in a hot air oven maintained at 50 °C until they became crispy and dry. The dried leaves were then grinded in mortar and pastel to dry powder. The powder was then stored in air tight sealed container until further use. For ethanolic extract formation, 0.1g dried powder was dissolved in 20 ml of ethanol and kept for 30 min which was then filtered. This process of extraction was practiced freshly for each study.

**Physical examination**

The colour and constituency of the dried powder were monitored regularly. pH of the ethanolic leaf extract was determined using a digital pH meter before use by measuring the pH of a standard buffer solution of pH 9 and another of pH 3 respectively.

**Chemical examination (Qualitative screening)**

Phytocomponents like saponins, tannins and phenols, gum and mucilage and flavonoids etc. were qualitatively estimated by using qualitative analysis of the phytocomponents of ethanolic leaves extracts of *Coriandrum sativum* revealed the presence of tannins and phenols, gum and mucilage and flavonoids etc. Saponin was found to be absent.

**RESULTS**

**Table 1: Qualitative analysis of the phytoconstituents of ethanolic leaves extract of Coriandrum sativum**

| Phytoconstituents          | Method Used              | Results |
|----------------------------|--------------------------|---------|
| Saponins                   | Foam method              | -       |
| Tannins and Phenols        | Ferric chloride method   | +       |
| Gum and Mucilage           | Alcohol method           | +       |
| Flavonoids                 | Sodium hydroxide method  | +       |

+ : Present; - : Absent

Qualitative analysis of the phytoconstituents of ethanolic leaves extracts of *Coriandrum sativum* revealed the presence of tannins and phenols, gum and mucilage and flavonoids. Saponin was found to be absent.

Our studies reveals that there was no significant alteration in the level of lipid peroxidation or activity of the antioxidant enzyme, catalase in goat heart when the tissues were treated *in vitro* with various doses of *Coriandrum sativum* (10 µl, 20 µl, 30 µl, 40 µl(50 µl) [table 2].
Table 2: Effect of various doses of ethanolic extract of *Coriandrum sativum* on the level of lipid peroxidation and catalase activity of goat heart

| Parameters                      | Control  | CsEth 10 | CsEth 20 | CsEth 30 | CsEth 40 | CsEth 50 |
|--------------------------------|----------|----------|----------|----------|----------|----------|
| Level of lipid peroxidation [nmoles of TBARS per mg protein] | 13.97±1.0234 | 13.78±1.0432 | 14.18±1.0232 | 13.71±1.0487 | 14.01±1.0384 | 14.14±1.0329 |
| Catalase activity [µmoles of H$_2$O$_2$ consumed per minute per mg protein] | 60.54±0.4942 | 61.55±0.15899 | 62.34±0.2127 | 62.55±0.3150 | 62.60±0.1159 | 62.76±0.1822 |

n= 6, Values are mean+SE

Our studies reveal that increasing doses of metanil yellow caused an increase in the level of lipid peroxidation in a dose-response manner in goat heart *in vitro*. Metanil yellow at a dose of 35 µl (concentration: 1 mg/ml) caused a significant increase in the level of lipid peroxidation in goat heart *in vitro* compared to that of control (*P<0.01 Vs. Control) [Fig. 1].

![Fig. 1: Effects of various doses of metanil yellow on lipid peroxidation level in heart tissue of goat (n=6) Values are mean+SE, *P<0.01 Vs. control](image1)

Our studies reveal that increasing doses of metanil yellow caused a gradual decrease in the activity of catalase in a dose-response manner in goat heart *in vitro*. Metanil yellow at a dose of 35 µl (concentration: 1 mg/ml) (MY35) caused a significant decrease in the activity of catalase in goat heart *in vitro* compared to that of control (*P<0.01 Vs. Control) [Fig. 2]. We used the dose of 35 µl (concentration: 1 mg/ml) of metanil yellow for the rest of our study.

![Fig. 2: Effects of various doses of metanil yellow on catalase activity in heart tissue of goat (n=6) Values are mean+SE, *P<0.01 Vs. control](image2)

Our studies reveal that metanil yellow at a dose of 35 µl (concentration: 1 mg/ml) caused a significant increase in the level of lipid peroxidation compared to that of control in goat heart *in vitro*. Metanil yellow at a dose of 35 µl (MY35) caused a significant decrease in the activity of catalase in goat heart *in vitro* compared to that of control (*P<0.01 Vs. Control) [Fig. 3]. We found that co-treatment of heart tissues of goat *in vitro* ethanolic extract of *Coriandrum sativum* (EthCs) at various doses (10 µl, 20 µl, 30 µl, 40 µl, 50 µl) protected the level of lipid peroxidation in goat heart from being increased in a dose response manner compared to that of metanil yellow treated heart. Our studies further reveal that co-treatment of goat heart tissue with ethanolic extract of *Coriandrum sativum* at the dose of 30 µl protected the level of lipid peroxidation from being increased in the cardiac tissue mediated by metanil yellow exposure at the dose of 35 µl (MY35) (concentration: 1 mg/ml).

The level of lipid peroxidation in the 35 µl (concentration: 1 mg/ml) metanil yellow and 30 µl ethanolic extract of *Coriandrum sativum* (MY35+EthCs30) co-treated tissue was found to be significantly lowered than that of metanil yellow exposed tissue (MY35) (**P<0.01** [Fig. 3, 5]).
Our studies reveal that metanil yellow at a dose of 35 µl (concentration: 1 mg/ml) caused a significant decrease in the activity of catalase enzyme in heart tissue of goat (n=6 each) compared to that of control. We found that co-treatment of heart tissues of goat with ethanolic extract of Coriandrum sativum (EthCs) at various doses (10 µl, 20 µl, 30 µl, 40 µl, 50 µl) protected the activity of catalase enzyme in goat heart from being increased in a dose response manner compared to that of metanil yellow treated heart. Our studies further reveal that co-treatment of goat heart tissue with ethanolic extract of Coriandrum sativum at the dose of 30 µl protected the activity of catalase enzyme from being decreased in the cardiac tissue mediated by metanil yellow exposure at the dose of 35 µl (concentration: 1 mg/ml).

The activity of catalase enzyme in the 35 µl (concentration: 1 mg/ml) metanil yellow and 30 µl ethanolic extract of Coriandrum sativum (MY35+EthCs30) co-treated tissue was found to be significantly higher than that of metanil yellow exposed tissue (MY35) (**P<0.01) [fig. 4, 6].
DISCUSSION

Catalase is a conjugative homotetrameric enzyme having Fe³⁺-centred heme group. It has a molecular weight of 64 kDa. Eukaryotic enzyme having both catalase and peroxidase activity. It is one of the chief subcellular antioxidant defence enzyme with the highest activity in liver and erythrocytes, followed by kidney and adipocytes, moderate in lungs and pancreas and heart in brain and heart. It is one of the key detoxifier of H₂O₂ converting the former into water and oxygen. It scavenges about 80% of H₂O₂ in cardiac myocytes yet its overall activity is low. This may partially account for the high sensitivity of the heart to oxidative insult [24]. Studies revealed that in ischemia-reperfusion injury, subsequent supplementation of catalase in perfusion fluid can efficiently detoxify H₂O₂ in the isolated heart [25].

Heavy metals induced cardiac toxicity is known to reduce catalase activity significantly [26]. Metanil yellow, when administered in single dose parenterally, induces P-450 and its dependent monooxygenases which is related it’s to hepatic metabolism and toxicity [27]. P-450 dependent monooxygenase is also adequate in the heart. This heme-thiolate enzyme is involved in the metabolism of environmentally toxic chemicals and endogenous substrates through oxidative, peroxidative and reductive changes and some of the subfamily of enzymes may be detrimental to cardiac function as it may induce the generation of ROS [28]. Metanil yellow exposure to isolated heart perhaps by some likely mechanism induces ROS production in the heart. A significant reduction in catalase activity in isolated goat heart exposed to metanil yellow in vitro may be attributed to excess free radical generation by metanil yellow [5] analogous to acute myocardial infarction which is a consequence of an excess of H₂O₂ and superoxide production resulting in decreased activity of catalase [29].

LPO activity in the metanil yellow exposed isolated hearts was observed to be significantly enhanced. LPO is approximated by the accumulation of malonic dialdehyde [30]. There occurs a spectrum of cellular alterations including membrane phospholipid peroxidation, thiol oxidation, utilization of major chain-breaking membrane anti-per oxidants. Further oxidative alterations of myocardial membrane polyunsaturated fatty acids are related to increased level of lipid peroxidation which may be a probable manifestation of progression of myocardial infarction [31].

Amongst the various mechanism involved in free radical induced lipid peroxidation followed by myocardial ischaemia, a deterioration of antioxidant system in the myocardium is suggested [32]. In the present study, increased lipid peroxidation may be attributed to decreased catalase activity since the available literature described catalase as a significant enzyme in cardiac myocytes in terms of its low content but highest activity in scavenging hydrogen peroxides which otherwise generates lipid peroxides and lipid hydroperoxides.

The findings of the present work also suggest that metanil yellow is cardiotoxic and may be an inducer of myocardial infarction mediated through oxidative stress. Cardioprotection by phytochemicals or herbal medicine is widely accepted trend and is already in use recently. Many plants are known to be a good source of carotenoids, triterpenes, flavonoids, and alkaloids that are potent to combat free radical mediated cardiac toxicity including myocardial infarction and ischemic heart diseases [33]. Some of such commonly listed plants are Terminalia arjuna, Andrographis paniculate, Citrus aurantium, Vitis Vinifera, Cocos nucifera, Zingiber officinale, Allium cepa, Carcuma longa, Terminalia chebula, Azadirachta indica etc and many others [32]. Coriandrum sativum, however, is not enlisted. Our observations showed that ethanolic leaf extract of Coriandrum has a good potential to counteract metanil yellow lipid peroxidative damage to cardiac tissue and restoring the normal activity of catalase. It has already been admitted that plants containing tannins, polyphenols and flavonoids have cardioprotective action [34]. Of the various phytoconstituent, qualitative estimation of ethanolic leaf extract of Coriandrum has shown the presence of a spectrum of compounds having antioxidant potential. Amongst such compounds phenols have been established as a good inhibitor of lipid peroxidation in vitro studies [35]. Polyphenols and tannins are competent metal chelators and their high redox potentials bestow them with free radical quenching and hydrogen donating potential [36]. Flavonoids and tannins can also check superoxide radical generation [37]. Further, it has been reported that flavonoid content in tea leaf extract may be accountable for the decrease in lipid peroxidation and restoration of catalase activity in cadmium-induced oxidatively stressed rat [38]. The observations of the present study mimic such similar findings and it may be thus summed phenolic constituents, flavonoids and tannins present in Coriandrum leaves may be good detoxifiers of free radical mediated peroxidative damage in goat heart exposed to metanil yellow and efficiently refurbish normal catalase activity in the heart.

CONCLUSION

Thus it may be concluded that altered metabolic pattern induced by metanil yellow in the isolated hircine heart may induce cardiotoxicity by the generation of ROS which includes superoxide anion radical followed by spawning of excess hydroxyradical that impinges normal catalase activity subsequently leading to peroxidative damage to lipids of isolated heart in vitro. Ethanol leaf extract of Coriandrum sativum may alleviate such free radical mediated cardiotoxicity because of the presence of indigenous phytochemicals like tannins, flavonoids and phenols that quench the free radicals with the beneficial effect of restoring catalase activity in vitro. Thus the study may indicate that the leaf extract of this plant might be promising in the development of new drugs to treat various diseases including cardiac ailments.
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Dr. SG and Dr. DG designed and conceived the study and analyzed the findings and together composed the manuscript. RND and SH performed the experiments and calculated and arranged the data. Finally, all authors read and approved the manuscript.

CONFLICT OF INTERESTS

Declared none

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