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PROTECTIVE EFFECT OF METHANOLIC LEAVES EXTRACT OF *CORIANDRUM SATIVUM* AGAINST METANIL YELLOW INDUCED LIPID PEROXIDATION IN GOAT LIVER: AN *IN VITRO* STUDY

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Abstract: Metanil yellow is an artificial colourant used to add colour to our foods . *Coriandrum sativu*m also known as 'dhania patta' is a popular spice herb and has some uses in folk medicine. The aim of our study is to find the protective role of methanolic extract of *Coriandrum sativum* (MECs) on Metanil yellow induced lipid peroxidation in liver tissue of goat, *in vitro*. The objectives of our study are to find the basic phytoconstituents of methanolic extract of *Coriandrum sativum* leaves, to find if an increasing dose of methanolic extract of *Coriandrum sativum* leaves has any effect on the level of lipid peroxidation in goat liver tissue, *in vitro, t*o find the effect of increasing dose of Metanil Yellow on the level of lipid peroxidation in goat liver tissue, *in vitro,* to find the dose of Metanil Yellow that causes significant increase in the level of lipid peroxidation in goat liver tissue, *in vitro, t*o find the effect of increasing dose of methanolic extract of *Coriandrum sativum* leaves, if any, on the Metanil yellow induced increased level of lipid peroxidation in goat liver tissue, *in vitro, t*o find the effective dose of methanolic extract of *Coriandrum sativum* leaves that protects against Metanil yellow induced increased level of lipid peroxidation in goat liver tissue, *in vitro.*

Key Words: *Coriandrum sativum,* lipid peroxidation, Metanil yellow, methanolic extract, liver, *in vitro*

INTRODUCTION

Now a days we are highly addicted and attracted to colourful and readymade food (fast foods), street foods etc. Foods containing added colours include candy and confections; bakery goods; soft drinks; cereals; dairy products such as butter, ice cream, and sherbet; margarine; snacks; jams and jellies; and desert powder. These colours are chemical in nature and have adverse health effects. They are also not good for our digestive system and also for our health. Those causes various problems like stomach problem, stomqach ache, liver damage, cardiac problems etc. Generally food adulteration is a offensive act but studies reveal that both the unorganized and organized sectors use different kinds of synthetic colours to process food items like ladoo, besan.orange or yellow colour sweets etc. Liver is one of the most important organs in our body. It is the largest digestive gland, highly purfused and a very important site of metabolism and enzymatic activities . If our liver is affected adversely, it will directly affect our well being [1]. Coriander leaves are popular spice herb in India and is used in continental as well as Eouropean cuisines. They have wonderful aroma and adds to the flavor of food. We have evaluated the phytocontents of these leaves and have studied if the methanolic extract of leaves of coriander leaves (*Coriandrum sativum*) have any effect on Metanil yellow induced lipid peroxidation in goat liver tissue.

The liver is the largest gland inside the body. It is located behind the ribs in the upper right-hand portion of the abdomen. Shaped like a triangle, the liver is dark reddish-brown and consists of two main lobes. There are over 300 billion specialized cells in the liver that are connected by a well organized system of bile ducts and blood vessels called the biliary system. The liver is such an important organ that we can survive only one or two days if it shuts down—if the liver fails, your body will fail, too. Fortunately, the liver can function even when up to 75% of it is diseased or removed. This is because it has the amazing ability to create new liver tissue (i.e. it

can regenerate itself) from healthy liver cells that still exist. Some of the most important functions that the liver performs are that it stores vitamins, sugar and iron to help give your body energy, controls the production and removal of cholesterol, clears our blood of waste products, drugs, and other poisonous substances, makes clotting factors to stop excessive bleeding after cuts or injuries, produces immune factors and removes bacteria from the bloodstream to combat infection, releases a substance called "bile" to help digest food and absorb important nutrients [2].

Metanil Yellow, a monosodium salt of 3 [[4(Phenylamino)phenyl]azo] benzene-sulfonic acid. In aqueous solution at low pH, methyl yellow appears red. Between pH 2.9 and 4.0, methyl yellow undergoes a transition, to become yellow above pH 4.0. It is a principal non-permitted food colour used extensively in India. The effect of long-term consumption of metanil yellow on the developing and adult brain causes neurotoxicity [3]. Metanil Yellow has mainly uses as food adulterant, in printing on paper, dyeing on fibres, silk, Wool, nylon, silk, paper, ink, aluminum, detergent, wood, fur, cosmetics, biological stain etc.

Coriander is an annual herb and, according to the climatic conditions, is cultivated as a summer or winter annual crop. Coriandrum which also called Dhania, is a green herb, used as spice and vegetable, it's green herb has a specific flavor, the green herb is also consumed on a large scale in India, in daily life we use its raw leaf , the whole herb is used in various cases. It also shows some antifungal activity and has use in Ayurvedachary and folk medicine.

METHODS AND MATERIALS

Preparation of an methanolic extract of the Coriandrum leaves

The leaves of Coriandrum were collected, separated, washed thoroughly in normal tap water and kept at room temperature for an hour on blotting paper to soak any excess water. The leaves were then dried in a hot air oven at 50 ^{o} Celsius for one and a half hour till they were dry and crispy and crushed into a coarse dust with mortar and pestle. Then they were filtered and the fine dust were stored in air tight Tarson bottles at room temperature until further use.

For aqueous extract preparation, the dried leaves dusts were soaked for 30 minutes in methanol (0.1g per 20 ml), filtered through loin cloth (fine cotton cloth) and the filtrate was used for our studies. Each time the leaves dust were soaked in methanol and filtered for fresh use, as per required.

Colour and consistency

The colour and consistency of the leaves dust was noted routinely.

Determination of relative leaf water content

Fresh weight (FW) of the leaves was measured and then the leaves were placed in 9 cm Petri dish containing distilled water. They were maintained for 24 h at 4°C in the dark. The leaves were then blotted dry and the turgor fresh weight (TW) was determined. Thereafter, the leaves were dried at 70°C for 24 h. Finally, the dry weight (DW) was measured [4].The relative water content was calculated by using the equation:

RWC (%) = [(W-DW) / (TW-DW)] x 100

Where,

W – sample fresh weight

TW – sample turgid weight

DW – sample dry weight.

pH of the extract

The methanolic extract was prepared and its pH was estimated using a digital pH meter which was checked before use by measuring the pH of a standard buffer solution of pH 9 and another of pH 3 respectively.

Qualitative screening of some of the phytochemical constituents of the methanolic extract of *Coriandrum sativum*

Saponins

About 1ml of the leaves dust was dissolved in 20ml of double distilled water and was shaken in a graduated cylinder for 15 minutes [5,6].

Tannins and phenols

A small quantity of the extract was taken and a 5% ferric chloride solution or a 1% gelatin solution or a 10% lead acetate solution was added to it [6].

Alkaloids

The extract was treated with dilute H_2SO_4 and filtered. The aliquot of the filtrate was tested separately with Mayer's, Dragendroff's, Hager's and Wagner's reagents [6].

Phytosterol and terpenes

The extract was treated with Lieberman Burchard reagent under suitable conditions [6].

Gum and mucilage

Ten ml of the extract was slowly added to 25ml of absolute alcohol under constant stirring [6].

Coumarin

To a 0.25 ml of leaves extract, 1.1% sodium carbonate was added, the mixture heated to almost boiling in water bath for few minutes, cooled slowly and to it 0.5 ml of diazonium solution was added and the volume was made up to 5 ml with double distilled water [7].

Flavonoids

The extract was treated with one ml of aqueous NaOH. The appearance of a yellow colour in the solution which becomes colourless on addition of HCl indicates the presence of flavonoids [6].

Preparation of tissue homogenate

A 10% tissue homogenate of liver was prepared (mostly with the fresh tissues) in ice cold 0.1Mphosphate buffer (ph 7.4) using a Potter-Elvehjem homogenizer.The homogenates, thus prepared, were used for further biochemical analysis.

***In vitro* incubation**

The homogenized tissues were incubated with MECs in phosphate buffer medium at different concentrations . In another set of experiment, homogenized tissues were incubated with Metanil yellow at various concentrations. In another the tissue homogenates were co-incubated with a particular dose of metanil yellow (which was found to be the significant dose from the earlier experiment) and various doses of the methanolic extract of *Coriandrum sativum*. Next , we set up an experiment with only homogenate in phosphate buffer (control), homogenate with the effective dose of MECs (positive control, the dose found from the earlier experiment), homogenate with only Metanil yellow in phosphate buffer solution , and in another with one we co-incubated the homogenate with effective dose of MECs and significant dose of Metanil yellow. The incubations were carried out at 37oC for 30 minutes . After the incubation was over, the aliquot were used for measurement of level of lipid peroxidation and protein estimation.

Measurement of lipid peroxidation (LPO) level

Level of lipid peroxidation (LPO) in the incubated reaction mixtures were determined as thiobarbituric acid reactive substances (TBARS) according to the method of Buege and Aust [8] with some modification [9]. Briefly, the homogenate was mixed with thiobarbituric acid–trichloro acetic acid hydrochloric acid (TBA–TCA) reagent with thorough shaking and heated for 20 min at 80^0C. The samples were then cooled to room temperature. The absorbance of the pink chromogen present in the clear supernatant after centrifugation at 1200 g for 10 min at room temperature was measured at 532 nm using a UV–Vis spectrophotometer (Bio-Rad, Hercules, CA, USA). Tetraethoxypropane (TEP) was used as standard. Values were expressed as nmoles of TBARS/mg protein.

Protein estimation

The protein concentration of the samples were estimated according to the method of Lowry et al. [10].

Statistical evaluation

Data were expressed as mean ± SE. Microcal Origin Version 7.0, a computer programme was used to compute statistical analysis. Significance of mean values of different parameters between the treatment groups were analyzed using one way analysis of variances (ANOVA) after ascertaining the homogeneity of variances between the treatment groups.

RESULTS

Colour and consistency

The colour of the leaves dust was green and its consistency was dry and powdery.

Relative leaf water content

The relative water content [RWC (%)] of the leaves was found to be 60.1 ± 0.76.

pH of the extract

The pH of the aqueous extract of the leaves was found to be 7.08.

Qualitative assessment of phytochemical constituents of the *Coriandrum sativum* leaves extract

The results of the qualitative assessment of the phytochemical constituent of the methanolic leaves extract of ***Coriandrum sativum*** are shown in **Table 1.**

Table: 1. Qualitative phytochemical screening analysis of methanolic leaves extract of *Coriandrum sativum*

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S.no	Phytochemical	Qualitative analysis	Results
			Methanol extract
5.	Saponins	Foam test	-
6.	Gum and Mucilage	Alcohol test	-
7.	Phenols	Lead acetate test	++
8.	Tannins	Ferric chloride test	++
9.	Flavanoids	Sodium hydroxide test	++

++: moderately present, +: Low, -: absent.

Effects of various doses of methanolic extract of leaves of *Coriandrum sativum* (CsMet) on level of lipid peroxidation in liver tissue of goat

Our study reveals that methnolic extract of leaves of *Coriandrum sativum* (Dhania patta) (0.1g per 20 ml)] when used in increasing concentration [10, 20, 30 and 40 µl] causes no alteration in the level of lipid peroxidation in goat liver *in-vitro* (Fig 1) compared to control.

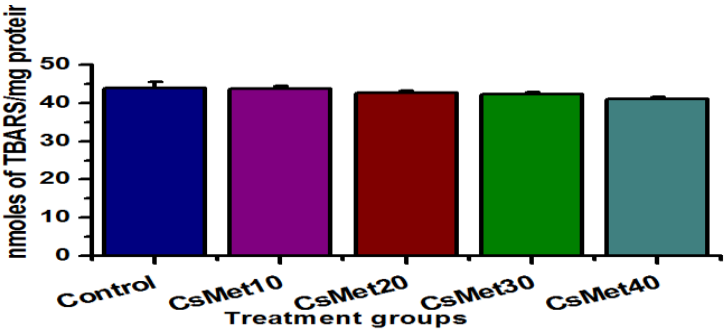


Fig.1. Effect of various doses of methanolic extract of leaves of *Coriandrum sativum* (CsMet) on level of lipid peroxidation in liver tissue of goat

Effect of various doses of Metanil yellow (MY) on level of lipid peroxidation in liver tissue of goat

We also found that goat liver tissue when treated *in vitro* with increasing doses of Metanil yellow [10, 25, 35 and 50 µl of Metanil Yellow (1mg per ml)], caused gradual increase in the level of lipid peroixidation in dose response manner indicating induction of

oxidative stress. The increase in the level of lipid peroxidation was observed to be significantly high (P<0.01Vs. Control) when goat liver homogenate was treated with 35µl of Metanil yellow (1mg/ml) (Fig. 2.).

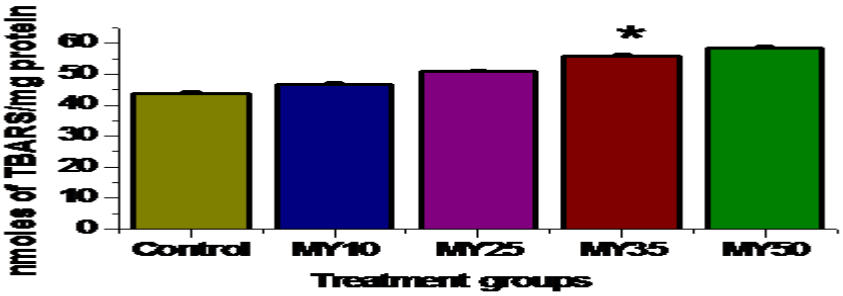


Fig.2. Effect of various doses of Metanil yellow (MY) on level of lipid peroxidation in liver tissue of goat

Effect of various doses of methanolic extract of leaves of *Coriandrum sativum* (CsMet) against Metanil yellow (MY) induced level of lipid peroxidation in liver tissue of goat

Goat liver homogenate when co-treated *in-vitro* with increasing dose of methanolic leaves extract of *Coriandrum sativum* [10, 20, 30 and 40 µl of methanolic extract of *Coriandrum sativum* (0.1g per 20 ml)] and Metanil yellow at the dose of 35µl (1 mg/ml), caused a gradual decrease in the level of lipid peroxidation in a dose response manner. The inhibition of metanil yellow induced lipid peroxidation in goat liver tissue was observed to be significant when the liver tissue homogenate was co-treated with methanol leaves extract of *Coriandrum sativum* at 30µl dose (Fig3).

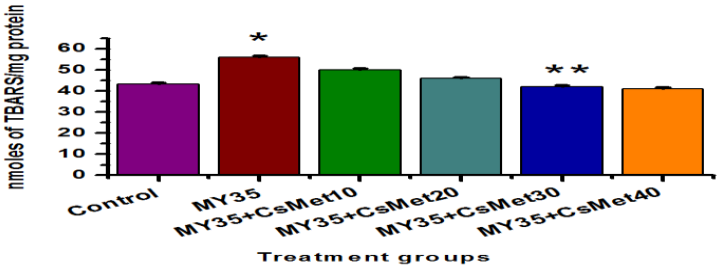


Fig.3. Effect of various doses of methanolic extract of leaves of *Coriandrum sativum* (CsMet) against Metanil yellow (MY) induced level of lipid peroxidation in liver tissue of goat (*P<0.01 Vs. Control; **P<0.01 Vs. My 35)

Effects of 30 µl of methanolic extract of leaves of *Coriandrum sativum* (CsMet 30) against 35 µl Metanil yellow (MY35) induced level of lipid peroxidation in liver tissue of goat

We observed that 30 µl of methanolic extract of leaves of *Coriandrum sativum* (CsMet 30) significantly protects against 35 µl Metanil yellow (MY35) induced lipid peroxidation in liver tissue of goat (fig. 4.).

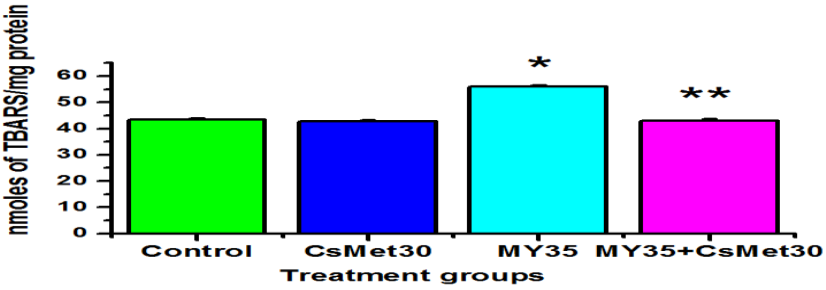


Fig.4. Effect of 30 µl of methanolic extract of leaves of *Coriandrum sativum* (CsMet 30) against 35 µl Metanil yellow (MY35) induced level of lipid peroxidation in liver tissue of goat (*P<0.01 Vs. Control; **P<0.01 Vs. MY 35)

DISCUSSION

Liver is a highly purfused glandular organ of our body. It is one of the most important organs of our body and it is the main site for various metabolic processes. The liver receives the first load of any toxicant absorbed from our food. Hence, liver faces the maximum, concentration of the toxicants those enter out body through mouth and digestive system. Lipid peroxidation is a spontaneous natural process, through this process by which free radicals are produced inside our cell and cause cell membrane damage. Liver is a very efficient organ and can handle the stress incurred by various toxicants and xenobiotics. Liver is also an important excretory organ and helps to eliminate various toxicants from our body. Metanil Yellow being a food colourant finds its way in our body through food only. It is absorbed along with other various food components from our digestive system and enters the liver. The toxic effect of Metanil yellow is primarily accumulative. It gets accumulated in the liver and induces generation of free radicals who in turn causes oxidative stress mediated damage to hepatic cells. Structural damage of hepatic cells lead to functional compromisation of the liver. Lipid peroxidation is the oxidative degradation of lipids, primarily membrane lipids. Free radicals induce this chain reaction of degration of cellular and organells' membrane and causes damage of cells. Metanil yellow has certain toxic effects on various organs and organ system in human and other experimental animals. The effects are being observed in cases of long-term consumption of Metanil yellow. The chemical compound has neurotoxic impact. It has been found to causes adverse effect on the developing and adult brain and those studies were conducted using using Wistar rats [3]. Metanil yellow as we have found induces lipid peroxidation and causes an increase in the level of lipid peroxidation. This indicates that Metanil Yellow induces damage of cellular membrane integrity and thus damages liver cells. Further we have observed that Metanil yellow causes an increase in the level of lipid peroxidation in a dose response manner. Lipid peroxidation is considered as the main molecular mechanisms involved in the oxidative damage to cell structures and in the toxicity process that lead to cell death [11].

We have observed that the situation of Metanil yellow enhanced lipidperoxidation in goat hepatic tissue is mitigated when treated with methanolic extract of *Coriandrum sativum*.

Coriandrum sativum is a very popular spice herb, used to add extra flavor and delicacy to various dishes. The leaves are the prime ingredients which are used in multiple ways in different Indian, Western and Continental food preparations [12,13]. It is already known and established scientifically that people who consume more vegetables and fruits show significantly good health compared to those who consume the least [13] . *Coriandrum sativum* is an important source of chemicals of α-pinene, γ-terpinene, limonene and ρ-cymene together with various nonlinalool alcohols and esters.

Coriander is knwn fr its use as Digestive, Astringent, Liver stimulant, Anthelmintic, Diuretric, Dyspeptic, Anti pyretic, Antiinflammatory. The essential oil of Coriander exhibit strong antifungal activity at very low concentration [13-16]. Phytochemicals are compound other than vitamins and minerals, which are present in plants and are known to be biologically active. Phytochemicals have been shown to possess antioxidant activity [12].

Our studies have revealed presence of various phytochemicals like alkaloids, polyphenols and flavonoids in the methanolic extract of *Coriandrum sativum*. Those phytochemicals are known to have potent antioxidant activity.

Tannins are plant polyphenols and they are made up of a very diverse group of oligomers and polymers. The wide distribution of tannins in the plant kingdom is somehow related to their antioxidant and antimicrobial properties [17].

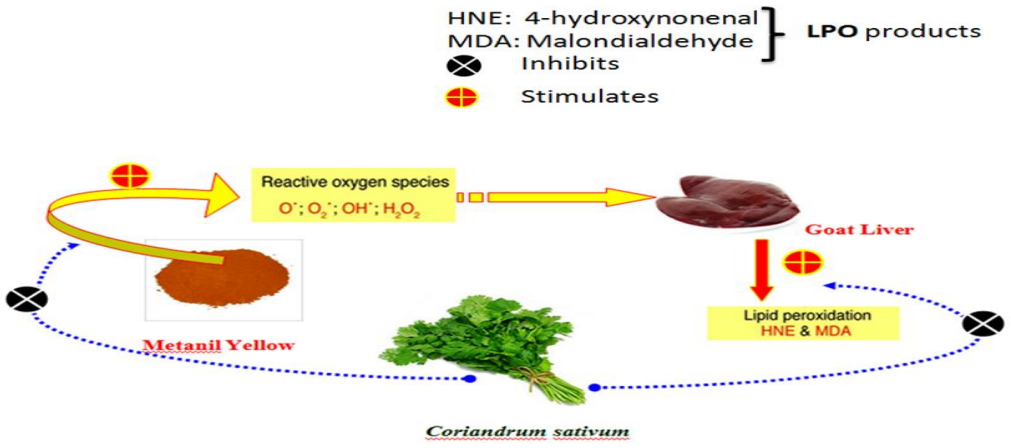
The ability of phenolic compounds to quench free radicals arises because of both their their delocalized π-electrons (ability to transfer electrons while remaining relatively stable) and acidity (ability to donate protons) and characteristic of benzene rings [17]. The thousands of phenolic phytochemicals can be organized into two main groups: polyphenols and flavonoids [18].

Polyphenols are a broad family of naturally-occurring bio-active nutrients. They can be further divided into sub-groups i.e., bioflavonoids, anthocyanins and proanthocyanidins.

The mechanism of action of antioxidants have been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity and radical scavenging [19, 20].We have found that methanolic extract of *Coriandrum sativum* contains certains potent antioxidants and when this extract is used, protects against Metanil yellow induced lipid peroxidation in goat liver tissue *in vitro*. Thus we can think of a pharmaceutical formulation from methanolic extract of *Coriandrum sativum* with hepatoprotective potential against Metanil yellow induced hepatic damage.

CONCLUSION

Our studies reveal that methanolic extract of *Coriandrum sativum* contain certain phytochemicals which are antioxidant in nature and the extract has protective effect on Metanil yellow induced hepatotoxicity in goat liver, *in vitro* (Scheme 1). We have found the effective dose of Metanil yellow, *in vitro* , which causes significant enhancement in the level of lipid peroxidation in hircine hepatic tissue . We have also found the effective dose of methanolic extract of *Coriandrum sativum* which protects against Metanil yellow induced lipid peroxidation in goat liver tissue *in vitro*. Thus we have found the dose of methanolic extract of *Coriandrum sativum* which imposes protective effect on Metanil yellow induced damage in liver tissue of goat, *in vitro*. Phytochemical constituents of methanolic extract of *Coriandrum sativum* are thus the key players in the hepatoprotective potential of the extract. Compound from herbal sources have no or minimum side effects and thus much more safe and acceptable in preparation of drug formulations. We can thus look ahead for a potent hepatoprotective drug formulation from methanolic extract of *Coriandrum sativum* against Metanil yellow induced lipidperoxidation mediated hepatic cellular damage.



Scheme.1. Protective effect of *Coriandrum sativum* (CsMet) on Meyanil Yellow induced increased level of lipid peroxidation in liver tissue of goat

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