



## Ameliorating efficacy of eugenol against metanil yellow induced toxicity in albino Wistar rats

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### ABSTRACT

Metanil yellow, an azo dye, is a non-permitted synthetic food colour used extensively in India and other developing countries as food additive. Present communication reports the toxic effects of metanil yellow on hepatic and kidney tissues and its amelioration by eugenol, vitamin E and vitamin C. Oral administration of metanil yellow in albino Wistar rats for 28 days caused elevation in serum enzymes (glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, alkaline phosphatase), and total bilirubin along with decline in albumin and total protein levels. At tissue level, activities of oxidative stress markers viz., superoxide dismutase, catalase and reduced glutathione in liver and kidney were reduced to about half while malondialdehyde level increased significantly under the influence of metanil yellow. Co-administration of eugenol/vitamin E/vitamin C in metanil yellow intoxicated rats exhibited considerable restoration of oxidative stress as well as hepatic and renal function markers in serum and tissues. The study revealed that eugenol has antioxidant, hepatoprotective and renoprotective activities.

### 1. Introduction

A number of food additives are widely used in food industries to enhance quality, colour and flavour of food products for centuries to increase consumer acceptability. They constitute about more than 25,000 compounds including food preservative and flavouring agents to make the food more palatable and tasteful (Hirschbruch and Torres, 1998; Toledo, 1999). It has been reported that the Indian population consumes 220 mg of synthetic food colours per year due to highly expensive natural product (Singh, 1997). Liver is an important organ for metabolic system of human being where it plays a vital role in regulating various physiological processes in our body and has capacity to detoxify toxic food additive substances including food preservatives, food colouring and food flavouring agents (Subramonium and Pushpangadan, 1999). Many authors have studied the metabolic, toxicological (Reyes et al., 1996; Tanaka, 2006; Zraly et al., 2006) and carcinogenic disorders (Sasaki et al., 2002) induced by the administration of specific food.

Metanil yellow (Myl) [sodium 3-[(4-anilinophenyl) azo] benzenesulfonate] is extensively used in the developing countries as a colorant in ice-creams, soft drinks and beverages, coating of turmeric, *laddu*,

spices etc. due to its orange-yellow colour. Although the use of Myl as a colorant is not permitted, it is still widely used as a colorant in many food industries. The information regarding basic mechanism of toxicity induced by Myl after ingestion through gastrointestinal tract is very scanty. Myl disrupts the gastric and intestinal secretions by causing necrosis in the glandular and columnar epithelium cells of the stomach and intestine (Ghosh et al., 2017). In humans it causes toxic methaemoglobinemia (Sachdeva et al., 1992), cyanosis, allergic dermatitis (Hausen, 1994), carcinogenesis (Ramachandani et al., 1997), mutagenesis (Gupta et al., 2003) and also enzymatic disorders (Das et al., 1997).

Myl metabolism is responsible for free radical generation which is responsible for liver injury as well as kidney damage. Hepatic diseases have become one of the major causes of morbidity and mortality all over the world. In spite of tremendous advances in modern medicine, there are hardly any reliable drugs that protect the liver and kidney from damage and help in regeneration of hepatic and renal cells. The mammalian cells have various enzymatic and non-enzymatic systems to prevent adverse effects of free radicals. Many active plant extracts are frequently utilized to treat a variety of clinical conditions (Yamabe et al., 2012; Kumar et al., 2014; Kumar and Pandey, 2015). Spices and

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aromatic vegetable materials of natural origin are used in food industries to enhance flavor and fragrance qualities of food materials. Moreover, they are also used as traditional medicine. Cinnamaldehyde has been shown to be effective against Myl induced hepatotoxicity (Sharma et al., 2018a). Some spices contain eugenol that has a wide range of applications in perfumes, flavorings, essential oils and in medicine due to presence of one hydroxy and one methoxy group which are directly attached to the ring (Sharma et al., 2016). Spices are good sources of natural antioxidant. Very low amount of eugenol is usually present in cinnamon bark but it is the major component of cinnamon leaf essential oil. It is also abundantly present in *Syzygium aromaticum* (clove). Eugenol belong to phenylpropanoid class of phytochemicals and has one hydroxy and one methoxy group which are directly attached to the ring (Gupta et al., 2003; Gruenwald et al., 2010). Some studies showed that cinnamon extracts and its constituents possess antimicrobial (Mishra et al., 2009), insecticidal (Yang et al., 2005), anticancer (Nishida et al., 2003), anti-inflammatory (Tung et al., 2008), antioxidant (Murcia et al., 2004) and cholesterol-lowering effects (Khan et al., 2003). Since eugenol possesses considerable antioxidant, lipoprotective and reducing abilities and therefore it may act as hepatoprotective agent (Sharma et al., 2016, 2017). Vitamin C and vitamin E have potent free radical scavenging ability and are used as antioxidant supplement during many clinical conditions. Liv52<sup>®</sup>, a hepatoprotective drug is often used in the treatment of hepatic disorders and is also used as standard drug for comparing the hepatoprotective activity of natural phytochemicals and other drug like molecules during studies. Current study reports the protective efficacy of eugenol, a natural phenylpropanoid compound present in spices against Myl induced toxicity and oxidative stress in albino Wistar rats by evaluating various biomarkers present in serum and tissues. In addition, protective efficacy of vitamin C and vitamin E against Myl induced toxicity have also been reported.

## 2. Materials and methods

### 2.1. Chemicals

Metanil yellow, eugenol, vitamin C and vitamin E were purchased from Sisco Research laboratory Pvt. Ltd. (SRL), Mumbai, India. Liv.52<sup>®</sup> was obtained from Himalaya Drug Company, Bangalore, India. All other chemicals and reagents used were of analytical grades and purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Lab animal feed was procured from Pranav Agro Industries Ltd., Sangli, Maharashtra, India.

### 2.2. Experimental animals

Healthy albino Wistar rats of either sex, approximately of same age (weight 150–200 g) were procured from IITR, Lucknow. They were kept in departmental animal house in well cross ( $23 \pm 2^\circ\text{C}$ ) with light and dark cycles of 12 h of 1 week before and during experiments. Animals were provided with standard pellet diet and water was given *ad libitum*. The *in vivo* study was performed in accordance with the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the Institutional Animal Care Committee, Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

### 2.3. Experimental design

The rats were divided in seven groups I–VII ( $n = 6$ ). Toxicity was induced by the oral administration of Myl for four weeks. First group (I) animals served as normal control. Second group (II) animals were treated with Myl only (100 mg/kg body weight). Third group (III) animals were treated with Liv52 (50 mg/kg body weight) along with Myl. Fourth group (IV), fifth group (V) and sixth group (VI) were treated with vitamin C (ascorbic acid, 100 mg/kg body weight), vitamin E ( $\alpha$ -tocopherol, 100 mg/kg body weight) and eugenol (10 mg/kg body

weight) along with Myl, respectively. Seventh group (VII) animals were administered with olive oil only. It was treated as vehicle control. After completing 28 days dosing period all animals were sacrificed and the blood, liver, and kidney were collected for evaluation of biochemical parameters including enzymatic as well as non-enzymatic.

### 2.4. Blood collection and separation

Heart was punctured and 5 mL of blood was drawn. The part of the blood (2.5 mL) was allowed to clot and serum was separated at 2500 rpm for 15 min. Remaining blood (2.5 mL) was collected into heparinised vials and kept on ice for less than 1 h before processing. The sample was centrifuged at 3000 rpm for 15 min and plasma was collected. Plasma and serum were transferred into separate eppendorf tubes and stored at  $-70^\circ\text{C}$ , until analysis.

### 2.5. Assessment of hepatic and renal markers in serum

The biochemical parameters viz., serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin, urea and creatinine were assayed using commercially available kits (Erba Diagnostic Kits).

### 2.6. Assessment of enzymatic and non-enzymatic parameters in tissue homogenate

#### 2.6.1. Preparation of tissue homogenate

The 10% (w/v) homogenates of liver and kidney were prepared in 0.25 M sucrose solution and centrifuged at  $9000 \times g$  for 30 min at  $4-6^\circ\text{C}$ . The supernatant was separated by gentle decantation and used for assay of antioxidant enzymes and estimations of the other biochemical markers.

### 2.7. Estimation of malondialdehyde (MDA) in homogenate

Lipid peroxidation was measured in the cytosolic fraction of tissues by the method of Niehaus and Samuelsson (1968). Tissue homogenate prepared in Tris-HCl buffer (0.1 mL, pH 7.5) was treated with 2 mL of TBA-TCA-HCl reagent (thiobarbituric acid 0.37%, 0.25N HCl, and 15% TCA in 1:1:1 ratio) and tubes were placed in water bath for 15 min. Subsequently, the samples were cooled and centrifuged. Absorbance of the clear supernatant was measured against reference blank at 535 nm. The results were expressed as nmol MDA/mg protein using the extinction coefficient of  $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ .

### 2.8. Estimation of reduced glutathione in tissue homogenate

The reduced glutathione (GSH) content in tissue homogenates was determined by the method of Ellman et al. (1961). Briefly, the 250  $\mu\text{L}$  supernatant of tissue homogenate was mixed with 100  $\mu\text{L}$  of 6 mM DTNB, 300  $\mu\text{L}$  phosphate buffer (0.2 M, pH 8.0) and 50  $\mu\text{L}$  NaOH (0.3 M). The absorbance of the reaction mixture was measured at 412 nm. GSH was used as standard and its level was estimated using regression analysis. All the values were expressed as  $\mu\text{g mg}^{-1}$  protein.

### 2.9. Estimation of the activities of antioxidant enzymes

The activity of superoxide dismutase (SOD, E.C. 1.15.1.1) was measured by the method of Marklund and Marklund (1974). Absorbance of coloured complex involving pyrogallol auto-oxidation at 412 nm was measured for 3 min at the interval of 30 s with or without the enzyme protein. One unit of the enzyme activity was expressed as 50% inhibition of auto-oxidation of pyrogallol per minute. The catalase (CAT, E.C. 1.11.1.6) activity was measured according to the method of Beers and Sizer (1952) by measuring the decrease in the absorbance of  $\text{H}_2\text{O}_2$  consumption at 240 nm at the interval of 30 s for 3 min. One unit

of CAT activity was defined as micromoles of H<sub>2</sub>O<sub>2</sub> decomposed per min using molar absorbance of H<sub>2</sub>O<sub>2</sub> (43.6 M<sup>-1</sup>cm<sup>-1</sup>).

### 2.10. Determination of total protein

The protein content present in different samples was measured according to the method of Lowry et al. (1951) using BSA as a standard.

### 2.11. Histopathological evaluation of liver and kidney slices

Immediately following sacrifice of the rats, histological examinations were conducted on liver and kidney slices. Tissue biopsies from these organs were fixed in 10% formalin, processed and embedded in paraffin. The paraffin block was cut into 5 µm slices with a rotary microtome and stained with hematoxylin and eosin (Cardiff et al., 2014). The slides were examined under a microscope (Dewinter) at 40 × magnification for observing histopathological changes.

## 3. Results

### 3.1. Effect of eugenol, vitamin C and vitamin E on serum enzyme markers and bilirubin

The hepatoprotective activities of test compounds in Myl induced toxicity were assayed by measuring the level of enzymatic and non-enzymatic hepatic markers in serum. Oral administration of Myl in group II rats for twenty eight days produced liver damage as indicated by enhanced activities of serum enzymes viz., SGOT, SGPT and ALP.

Myl administration in rats for four weeks led to a significant increase in the levels of SGOT (149 IU/L), SGPT (50 IU/L), ALP (243.3 IU/L) and total bilirubin (1.38 mg/dl) in Group II animals. Co-administration of Liv52 (Group III), vitamin C (Group IV), Vitamin E (Group V) and eugenol (Group VI) along with Myl caused reduction in the level of serum biomarkers. Vitamin E exhibited comparatively better hepatoprotective efficacy. Best restorative efficacy on serum enzymatic parameters was observed in the positive control (Liv52 treated rats). Vitamin E and vitamin C caused 26–50% and 4–40% reduction in the level of serum enzymes, respectively. Test compound eugenol accounted for 22–32% reduction in the activities of SGOT, SGPT, and ALP as compared to Myl fed rats. Similarly eugenol/vitamin supplementation caused substantial reduction in the level of total bilirubin, bringing them to normal level. However, activity of eugenol was little less than the activity of vitamin E (Fig. 1). No significant difference was observed in vehicle control group as compared to normal rats.

### 3.2. Effect of eugenol, vitamin C and vitamin E on other serum markers

The level of albumin and total proteins significantly declined in the Myl treated rats (Group II) as compared to normal rats (Fig. 2). Co-administration of vitamin C and vitamin E along with Myl restored the level of serum albumin and total protein towards the normal values. Liv52 treated rats (positive control) exhibited substantial restorative efficacy on protein and albumin levels. Vitamin E caused restoration of albumin and total protein up to about 77% and 86% while vitamin C restored the level of albumin and total protein up to the 69% and 71%, respectively. Further eugenol also caused enhancement in total protein by 34%.

Oral administration of Myl in rats caused elevation in serum creatinine. However elevated level was still within the normal range. Co-administration of eugenol and vitamins/Liv52 with Myl exhibited marginal reduction in serum creatinine. Serum urea in all groups was also within normal range.

### 3.3. Effect of eugenol on antioxidant tissue markers

Oral administration of Myl caused a significant reduction in the

level of GSH coupled with elevated MDA levels in liver and kidney tissues as compared with the normal control (Table 1). Myl exposure (Group II) of tissues *in vivo* caused about 50% depletion of the glutathione content in liver (59.1 nM/mg protein) and kidney (56.3 nM/mg protein) homogenates. The rats treated with Liv52 (Group III) accounted for restoration of glutathione levels by elevating GSH content near normal values in liver and kidney tissues. Co-administration of eugenol/vitamin C/vitamin E along with Myl showed considerable augmentation in the level of GSH in both the tissues (Table 1). The percentage replenishment of the GSH level in both tissues after administration of eugenol (Group VI) and Liv52 (Group III) along with Myl were about 68–79% and 87–91%, respectively. Myl treatment caused about three times elevation in MDA level in both the tissues. Liv52 in group III brought down the MDA level in both tissues up to two folds (1.03–1.12 nM/mg protein). Eugenol accounted for reduction in MDA level in both the tissues. In normal rats (Group I) the MDA levels were found to be 0.5 nM/mg protein (liver) and 0.64 nM/mg protein (kidney).

### 3.4. Assessment of antioxidant enzymes in tissue homogenate

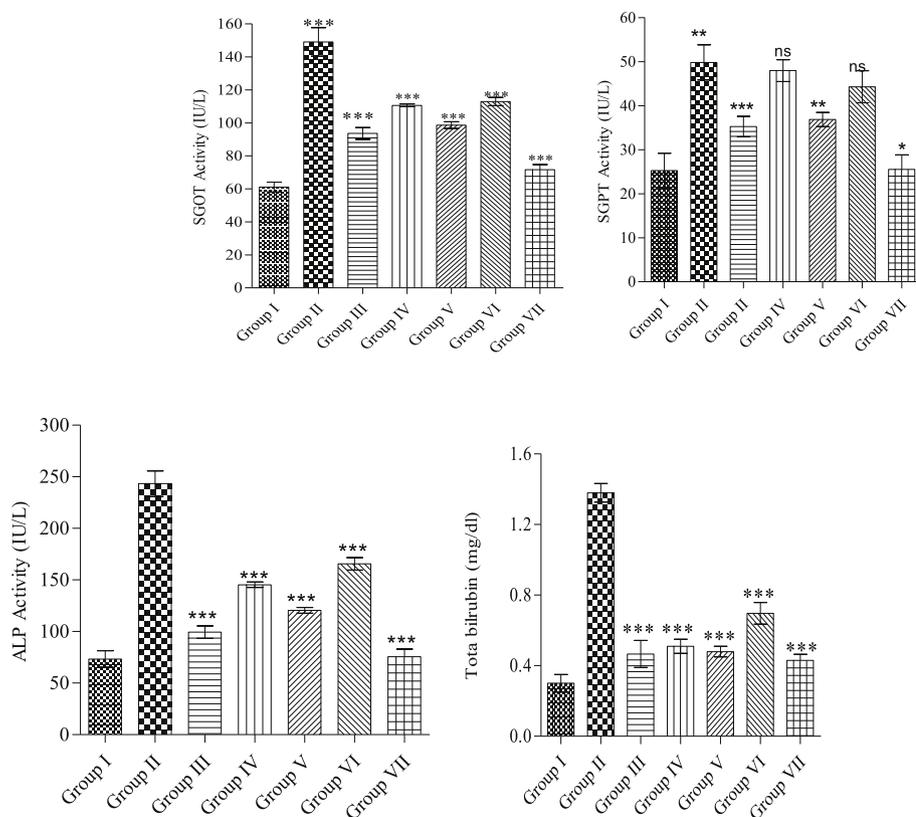
The activities of SOD and catalase were monitored in liver and kidney tissues of albino Wistar rats fed with Myl and test compounds. Enzyme activities are displayed in Table 1. Decreased enzyme activities were observed in liver and kidney of group II rats. In quantitative terms activities of SOD (17.60U/mg) and catalase (7.7 U/mg) observed in group I liver were reduced to about half (8.46 and 3.18 U/mg, respectively) under the influence of Myl. Liv52, in combination with Myl in liver was responsible for enhancing the activities of SOD and catalase to 70% and 81%, respectively of the normal values. Further co-administration of eugenol (Group VI) with Myl showed protective effect on liver by restoring the SOD enzyme activities up to 75% of the original value. Similar trend in enhancement of catalase activities after supplementation of Myl with Liv52, vitamin C, vitamin E and eugenol were also observed in liver and kidney (Table 1). In group III about 80% of the activity was observed for SOD and catalase showing appreciable protective efficacy of Liv52. Myl treatment caused a significant reduction in the activities of both antioxidant enzyme markers in liver and kidney homogenates.

### 3.5. Histopathological studies of eugenol fed liver and kidney tissues

Histopathological examination of liver specimen from normal control rats revealed normal hepatic architecture with distinct hepatic cells, sinusoidal spaces and central vein (Fig. 3A). Liver slices from Myl treated rats exhibited disarrangement of normal hepatic cells with centri-lobular necrosis, vascular and cellular degeneration, and inflammation. The activity of the reticulo-endothelial system inside the liver was much more prominent in the eugenol treated groups (Fig. 3C) than in the unprotected Myl treated group (Fig. 3B). Liver sections of rats treated with eugenol showed considerable reduction in necrosis and other histopathological changes induced by Myl (Fig. 3C). Further, renal intoxication with Myl was related with severe glomerular and tubulo-interstitial necrosis which was characterized by hydropic degeneration of the glomerular and tubular cells in comparison to normal rat kidney (Fig. 4 A, B). However, oral treatment with eugenol ameliorated renal histological anomalies showing minute vacuolization of epithelial lining tubules and endothelial linings (Fig. 4C).

## 4. Discussion

Liver is the largest and most vital organ of the human body. It plays crucial role in the metabolism of nutrients and is responsible for bio-transformation of drugs and chemicals. Thus it protects body against toxic foreign materials. Although the mechanism underlying xenobiotic induced liver injury is not completely understood, cytochrome p450



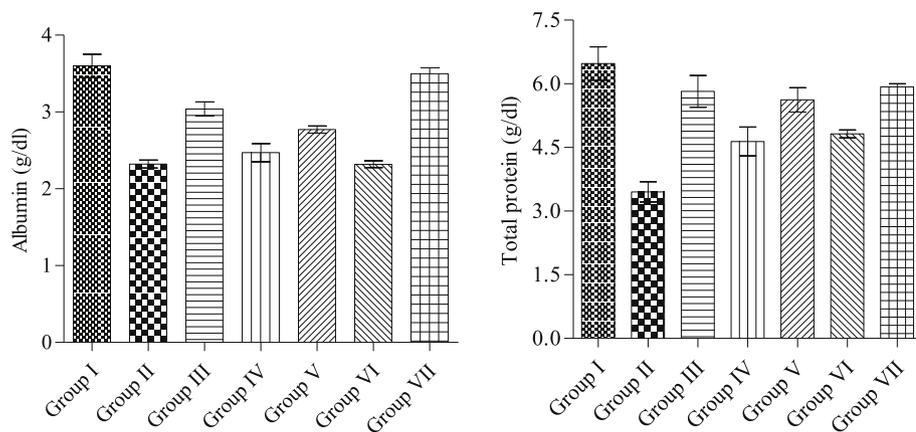
**Fig. 1.** Effect of eugenol (Eug), vitamin C (VC) and vitamin E (VE) on serum enzymes (SGOT, SGPT and ALP) and bilirubin in metanil yellow (Myl) induce toxicity in albino Wistar rats. [Enzyme activity was expressed as IU/L. The data represent mean  $\pm$  SD (n = 6; p < 0.001). Group I- Normal control; Group II- Myl treated; Group III- Liv52 + Myl; Group IV- Myl + VC; Group V- Myl + VE; Group Group VI- Myl + Eug; Group VII- vehicle control (olive oil treated)].

plays a key role in transformation of drug metabolites, and reactive metabolites can increase reactive oxygen species either through redox cycling or through GSH depletion (Park et al., 2014). The pathogenesis of liver and kidney disease is associated with free radical injury and oxidative stress, which could be partially attenuated by antioxidants and free radical scavengers.

In the present communication effect of eugenol, vitamin C and vitamin E on Myl induced oxidative damage in liver and kidney has been reported. The study showed that Myl inflicts hepatic damage as revealed by elevated levels of SGOT, SGPT and ALP. The rise in serum levels of enzymatic markers has been attributed to the damaged structural integrity of liver, because they are cytoplasmic in location and released into circulation after cellular damages (Adikwu and Nelson, 2012). Although SGOT and SGPT are markers of hepatocellular injury but their level is also elevated in cases of injury to other organs like kidney, heart and muscles (Valentine et al., 1990; Bain, 2003). The hepatoprotective drugs are used to maintain the structure and function of liver cells. Myl treated rats also showed elevation of total bilirubin

and diminution in the level of albumin and total protein in serum. Increased levels of bilirubin might also result due to decreased hepatic clearance and leads to jaundice and other hepatotoxicity symptoms (Saukkonen et al., 2006). Total protein is often slightly reduced but albumin to globulin ratio shows a sharp decline during hepatocellular injury. Also, this decrease can be attributed to the damage produced in the endoplasmic reticulum which results in the loss of cytochrome P450 leading to its functional failure with a decrease in protein synthesis. In earlier studies cinnamaldehyde, a phenylpropanoid has been shown to exert hepatoprotective and antioxidant activity in Myl fed rats (Sharma et al., 2018a). Similarly cinnamaldehyde supplementation has also been reported to provide renoprotection in food colour induced toxicity and oxidative stress in rat model (Sharma et al., 2018b). These studies highlighted the antioxidant and hepato/renoprotective abilities of phenylpropanoids. However in comparison with the results of earlier studies on other phenylpropanoids, the eugenol exhibited comparatively better biological activities in the current study.

Many researchers have studied the impact of food colouring agents



**Fig. 2.** Effect of eugenol (Eug), vitamin C (VC) and vitamin E (VE) on serum albumin and total protein (g/dl) in metanil yellow (Myl) fed albino Wistar rats. [The data represent mean  $\pm$  SD (n = 6; p < 0.001). Group I- Normal control; Group II- Myl treated; Group III- Liv52 + Myl; Group IV- Myl + VC; Group V- Myl + VE; Group Group VI- Myl + Eug; Group VII- vehicle control-olive oil treated].

**Table 1**

Effect of eugenol (Eug), vitamin C (VC) and vitamin E (VE) on level of GSH, MDA, SOD and catalase in liver and kidney tissue homogenate of Myl treated rats. GSH and MDA are represented as nM/mg protein while SOD and catalase are represented as U/mg. The data represent mean  $\pm$  SD (n = 6; p < 0.001). Group I- Normal control; Group II- Myl treated; Group III- Liv52 + Myl; Group IV- Myl + VC; Group V- Myl + VE; Group Group VI- Myl + Eug; Group VII- vehicle control-olive oil treated].

Groups	GSH		MDA		SOD		Catalase	
	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver
I	110.30 $\pm$ 1.52	118.1 $\pm$ 3.30	0.64 $\pm$ 0.03	0.545 $\pm$ 0.030	15.23 $\pm$ 0.55	17.60 $\pm$ 0.60	6.46 $\pm$ 0.40	7.78 $\pm$ 0.62
II	56.37 $\pm$ 1.51	59.27 $\pm$ 2.45	1.76 $\pm$ 0.01	1.867 $\pm$ 0.130	7.06 $\pm$ 0.30	8.64 $\pm$ 0.160	2.66 $\pm$ 0.15	3.18 $\pm$ 0.14
III	96.17 $\pm$ 1.87	107.20 $\pm$ 1.40	1.12 $\pm$ 0.02	1.033 $\pm$ 0.061	12.47 $\pm$ 1.193	12.39 $\pm$ 0.60	5.12 $\pm$ 0.08	6.40 $\pm$ 0.17
IV	75.47 $\pm$ 2.81	97.93 $\pm$ 2.67	1.46 $\pm$ 0.01	1.350 $\pm$ .088	9.86 $\pm$ 0.47	10.87 $\pm$ 1.00	3.73 $\pm$ 0.35	4.86 $\pm$ 0.20
V	82.67 $\pm$ 2.19	104.0 $\pm$ 3.65	1.34 $\pm$ 0.02	1.103 $\pm$ 0.075	11.7 $\pm$ 2.36	12.52 $\pm$ 0.88	4.93 $\pm$ 0.30	5.93 $\pm$ 0.24
VI	103.80 $\pm$ 2.49	92.53 $\pm$ 2.51	0.75 $\pm$ 0.02	1.50 $\pm$ 0.03	13.9 $\pm$ 0.80	9.86 $\pm$ 0.80	6.03 $\pm$ 0.30	4.84 $\pm$ 0.13
VII	112.60 $\pm$ 2.43	115.0 $\pm$ 2.43	0.623 $\pm$ 0.035	0.683 $\pm$ 0.035	14.32 $\pm$ 0.05	14.49 $\pm$ 0.65	6.85 $\pm$ 0.10	7.38 $\pm$ 0.10

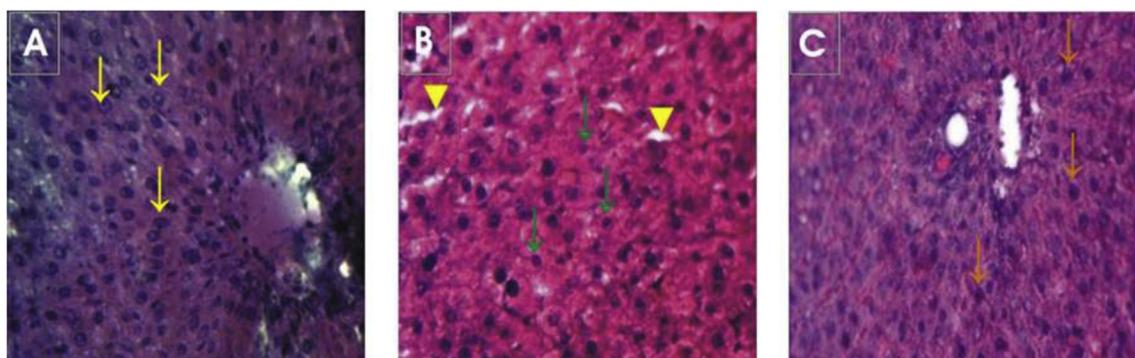
on the health of experimental animals (Tanaka, 2001; Amin et al., 2010; Feng et al., 2012). Myl intake is hazardous and causes lipid peroxidation in liver through free radical generation. In addition Myl also caused depletion of GSH, SOD and catalase coupled with elevation in MDA level in liver and kidney tissues. Supplementation of eugenol, vitamin C or vitamin E during Myl toxicity has shown reparative effect on hepatic, renal and antioxidant health. Hepatoprotective and renoprotective activity of vitamin C is attributed to its antioxidant property by decreasing lipid peroxidation either directly or indirectly through vitamin E regeneration. Vitamin E is fat soluble and is primarily located within the phospholipids bilayer of the cell membranes where it has a major biological role in protecting polyunsaturated fats and other components of the cell membranes from oxidation by free radicals (Al-Othman et al., 2011). Vitamin E supplementation has been reported to attenuate lipopolysaccharide induced liver damage by reducing levels of MDA, restoring the levels of GSH, SOD and catalase (Bharrhan et al., 2010). The ability of vitamin E to protect liver from chemical induced toxicity has also been reported (Khalifa et al., 2009; Frei, 2004). It has been demonstrated that vitamin E can directly reduce ROS production by interfering with the union between the membrane and the NADPH oxidase complex (Uboh et al., 2012). Comparative evaluation of vitamin E and vitamin C in Myl induced toxicity showed that vitamin E is a better hepatoprotective agent than vitamin C. The study is also supported by the earlier study (Uboh et al. 2012; Kumar et al., 2014).

GSH plays an important role in intracellular defense against ROS-induced oxidative damage. The balance between the GSH and oxidized glutathione, is a central component in maintaining cellular redox state. GSH plays an important role in diverse biological processes, including cell growth/division, regulation of sulfate transport, signal transduction, conjugation of metabolites, enzymatic regulation, synthesis of proteins and nucleic acids, synthesis of phytochelatins for metal chelation, detoxification of xenobiotics, and the expression of the stress

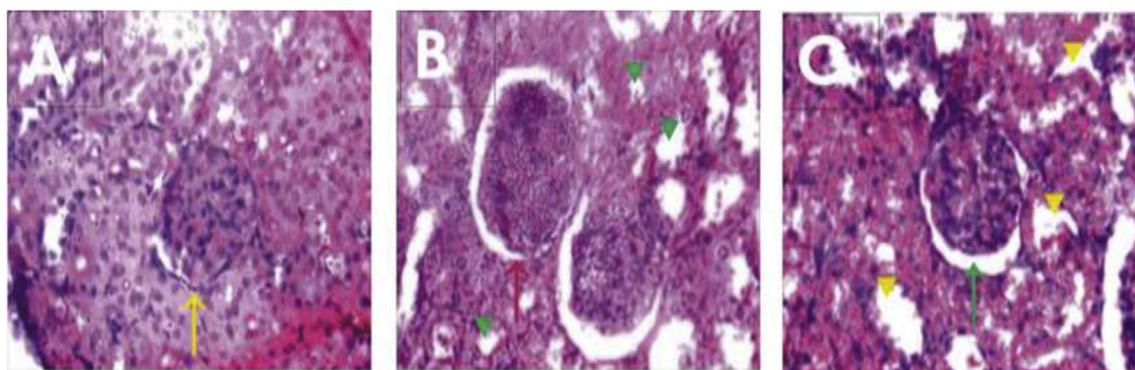
responsive genes [Foyer et al., 1997]. Under oxidative stress, GSH concentrations drops and redox state becomes more oxidized, leading to metabolic imbalance of the system (Rojas et al., 1994; Kumar and Pandey, 2015). Glutathione depletion is an indicator of tissue degeneration/damage. During study, eugenol, vitamin C and vitamin E exhibited regeneration of GSH, along with enhancement in SOD and catalase activities. Moreover, reduction in the MDA was also observed. GSH can function as a direct radical scavenger and can also stabilize membrane structure through the removal of acyl peroxides formed by lipid peroxidation. Thus, lipid peroxidation contributed to the depletion of tissue level of GSH. Level of MDA, the end product of lipid peroxidation, was found to be high indicating tissue damage which also shows the failure of antioxidant defense mechanisms against free radicals (Sharma et al., 2018a). Eugenol and vitamin E showed considerable protective activity against Myl inflicted toxicity. The liver sections of the rats treated with Myl and eugenol showed signs of protection as shown by the reduction/absence of inflammatory cells, vascular congestion and degeneration, cellular degeneration, necrosis and vacuoles (Fig. 3). Kidney slices of Myl treated rats showed hydropic degeneration of the glomerular and tubular cells when compared to normal rat kidney. However, oral treatments with eugenol ameliorated renal histology exhibiting minute vacuolization of epithelial lining tubules and endothelial linings (Fig. 4). Other phenylpropanoid viz., cinnamaldehyde has also been reported to exhibit antioxidant and renoprotective action during *in vitro* and *in vivo* studies (Sharma et al., 2017, 2018b). The results have revealed that eugenol provides protection against hepatotoxicity, renotoxicity and oxidative stress caused by Myl, a non-permitted food colour in rats.

## 5. Conclusion

Administration of Myl leads to oxidative stress coupled with hepatic and renal damage. It causes elevation in the levels of serum hepatic



**Fig. 3.** Histological section of liver. (A) Normal control (yellow arrow shows normal cells with normal nucleus), (B) Metanil yellow treated rats (green arrow shows shrinkage of nucleus and yellow arrow head shows lesion in liver) and (C) Eugenol treated rats (orange arrow shows recovery of cells as well as nuclear size in liver).



**Fig. 4.** Histological section of kidney slices. (A) Normal control (yellow arrow shows normal glomerulus), (B) Metanil yellow treated rats (red arrow shows shrinkage of glomerulus and green arrow head shows lesions in kidney), (C) Eugenol treated rats (green arrow shows recovery in the glomerulus from shrinkage and yellow arrow head shows recovery from lesion in kidney).

markers viz., SGOT, SGPT, ALP and total bilirubin along with decline in level of the albumin and total protein in serum. Further, it also causes depletion of antioxidant markers GSH, SOD and catalase and elevation in the level of MDA in liver and kidney tissue homogenate revealing that Myl induces tissue damages by free radicals mediated lipid peroxidation. Co-administration of eugenol, vitamin C and vitamin E helped in restoring the levels of various biomarkers towards the normal value in serum and tissue. The findings of the study showed that eugenol and vitamin E have considerable hepatoprotective, renoprotective and antioxidant efficacy against metanil yellow induced toxicity.

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#### Transparency document

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