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HEPATOPROTECTIVE EFFECTS OF EPIGALLOCATACHIN GALLATE VIA MITOCHONDRIAL PERMEABILITY TRANSITION PORE IN PARACETAMOL INDUCED HEPATOTOXICITY

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Abstract

Paracetamol (PCM) is a widely used as antipyretic throughout the world. Overdose of PCM is linked to many cases of hepatic failure and death in many countries. PCM is also used as an experimental model of hepatotoxicity screening. The protective efficacy of EGCG in PCM induced liver injury has assessed in present study. Wistar Rats were treated with 3 gm/kg body weight of PCM alone or with EGCG (40 mg/kg), Atractyloside (ATR) 5mg/kg a potent Mitochondrial Permeability Transition Pore (MPTP) opener is given and combination of EGCG+ATR pretreated PCM also given in one group. SGOT, SGPT, Bilirubin, TBARS and SOD in liver tissues had been predicted 6 hrs after PCM and EGCG treatment. EGCG was shown to be efficient in reducing aspartate transaminase (AST) and alanine transaminase (ALT) released from liver hepatocytes. EGCG also inhibited production of TBARS whereas supplementation with EGCG maintained near normal SOD level in liver of PCM treated rat. ATR treated also cause increase in liver injury as compared to the combination of EGCG+ATR pretreated PCM. It is noted that histopathological evaluation shown significantly reduced necrotic areas (47%) of liver samples in EGCG treated as compare to PCM control. EGCG treatment also prevents hepatocytes DNA fragmentation compared to PCM treated group. The survival test conducted confirmed that rat receiving PCM + EGCG are more resistant to the deleterious effect of PCM overdose than PCM alone. ATR indicates a change in liver cellular as examine to EGCG+ATR pretreated PCM. This examine showed the hepatoprotective activities of EGCG in PCM prompted liver damage in wistar rats and accordingly illustrated the advantage of non-stop intake of EGCG.

***Keywords*:** EGCG, Green tea, Hepatoprotective, liver

Introduction

Paracetamol (PCM) is likewise called acetaminophen. It's far extensively used as over the counter analgesic and antipyretic agent (Blough and Wu, 2011). The healing dose of PCM is more secure however its overdose is also considered because of its narrow healing index. Its overdose can result in hepatic and renal harm in both human beings and experimental animals (Varghese *et al.,* 2013). Liver is the main target organ for drug metabolism. PCM toxicity can occurs among patients with marked hepatic injury; however, PCM hepatotoxicity after overdose may be seen in animals too (Prescott, 2000). PCM metabolized via cytochrome p450 in various pathways like conjugation-sulfate, glucuronide (Cederbaum, 2015). Moreover 90% dose of PCM is metabolized through glucuronidation and sulfation pathways rest 5% metabolized via liver cytochrome- p450. It observed that PCM produced N-acetyl-p-benzoquinone imine (NAPQI) induced nephro and hepatotoxic metabolite. In the range of therapeutic, this NAPQI metabolite regulated/controlled by endogenous glutathione and excreted by kidney (Khuon, 2012; Mazaleuskaya *et al.,* 2015). The glucuronidation and sulfation pathways become saturated in case of acetaminophen overdose. The over activation of NAPQI followed by depression of antioxidant endogenous glutathione resulting hepatic cell death leads to liver damage (Mousah *et al.,* 2016). Therefore antioxidant compounds may be good sources for controlling effects of PCM toxicity (Dhibi *et al.,* 2014).

Green tea (*Camellia sinensis*) consists catechin derivatives including gallic acid (GA), epigallocatechin (EGC), epicatechin (EC), epigallocatechin 3-gallate (EGCG) and epicatechin 3-gallate (ECG), of which EGCG is a main active component (Bigelow and Cardelli, 2006; Du *et al.,* 2012). EGCG is the major component having good anti-oxidant quality because oxidative stress main culprit for liver injury (Singh *et al.,* 2011; Nugala *et al.,* 2012).

However EGCG is a major constituent and controlled the ROS generation (Saffari and Sadrzadeh, 2004), expression of PPARY, Interleukins formation TNF alpha and ATP generation (Oz *et al.,* 2013). It has been observed that EGCG up regulate the JAK/STAT (Tedeschi *et al.,* 2002), MAPK and PI3K/AKT pathways leading to successful protection of different organs against oxidative and inflammatory injuries in many experimental models (Huang *et al.,* 2014).

The present study investigates the protective effects of EGCG against paracetamol induced hepatotoxicity. For this purpose, EGCG was given to rats which were then treated with paracetamol histological preparations of the liver were examined with the light microscope in addition to measuring SGOT, SGPT and bilirubin.

Materials and Methods

Chemicals

Epigallocatachin Gallate (EGCG) was purchased from Sigma Aldrich Chandigarh. Atractyloside was purchased from Ausmasco Pvt. Ltd, China. Diagnostic kits used in study were purchased from Reckon Diagnostics Pvt. Ltd. Vadodara, India. Di-sodium hydrogen phosphate was purchased from Central Drug House (P) Ltd, New Delhi, India. Trichloroacetic acid (TCA), Thiobarbituric acid, Tris HCl and 5, 5'-Dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from Hi Media Laboratories Pvt. Ltd, Nashik. Adrenaline was purchased from Regain laboratories, Hisar. All other chemicals followed by reagents were employed in present study were of analytical grade.

EXPERIMENTAL DESIGN

Wistar rats of either sex, (150-200g) were employed in the present study and procured from Panacea Biotech. They were maintained on standard laboratory diet (Aashirwaad feeds Ltd., Chandigarh, India) and tap water ad libitum. Experimental animals were housed in the animal house of Rayat and Bahra Institute of Pharmacy and were maintained as per CPCSEA standard conditions. The experimental protocol of the study was duly approved by Institutional Animal Ethics Committee (IAEC), RBIP/IAEC/CPCSEA/2015/Protocol No: 16.

The dose of PCM (3g/kg) selected for present experimental study was standardized in our laboratory. Rats were administered Paracetamol (3g/kg, *p.o.*) on 3rd and 5th day of experimental protocol to produced hepatotoxicity. The selected dose did not cause any mortality and all the animals survived the six hours of experimental time frame during initial study. Each group comprised 5 animals.

Group 1: (Normal Control)

Animals in this group were treated with distilled water for 7 days of experiment protocol.

Group 2: (Atractyloside *per se*)

Animals in this group were treated with Atractyloside for 7 days of experiment protocol

Group 3: (Paracetamol Control)

Animals in this group were treated with Paracetamol on 3rd and 5th days of 7 days of experiment protocol.

Group 4: (EGCG Pretreated PCM)

Animals were treated with EGCG for 7 days in Paracetamol treated group.

Group 5: (EGCG + Atractyloside Pretreated PCM)

Animals in this group were treated with EGCG and Atractyloside for 7 days in Paracetamol treated control.

Table 1: Diagrammatic Representation of Experimental Protocol

GROUP	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8
GROUP 1	DW	DW	DW	DW	DW	DW	DW	SAC
GROUP 2	ATR	ATR	ATR	ATR	ATR	ATR	ATR	SAC
GROUP 3	---	---	P	----	P	---	----	SAC
GROUP 4	D	D	D+P	D	D+P	D	D	SAC
GROUP 5	D+ATR	D+ATR	D+ATR+P	D+ATR	D+ATR+P	D+ATR	D+ATR	SAC

DW-distilled water, ATR-Atractyloside, P-Paracetamol, D indicates Epigallocatachin Gallate (EGCG), SAC-sacrificed. Animals were sacrificed by cervical dislocation.

Hepatic Injury Evaluation Parameters

Biochemical hepatic injury evaluations

The SGOT, SGPT and Bilirubin estimated in the present study from the blood serum of animals by using biochemical enzymatic kits as a marker of liver injury.

Oxidative stress evaluations

TBARS [14] & SOD levels were measured by using tissue of animals.

Histopathological evaluations

The liver tissue of wistar rats were preserved in 10 % formalin solution and stain them by using haematoxylin, sectioned of liver (5µM) and seen with microscope (10X) to evaluate the histological variations.

Statistical Analysis

All data were presented as means ± SEM. Statistical analysis were performed using one way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p value < 0.05 were taken into consideration for determining significance.

Results

Effects of PCM and its combination on serum markers

To test the beneficial effect of EGCG in PCM induced liver injury, Paracetamol produced a significant elevation in the serum SGOT, SGPT and bilirubin when compared with untreated control rats. On the other hand, EGCG (40mg/kg, p.o.) for 7 days with PCM treated rats showed significant reduction in serum SGOT, SGPT and bilirubin level when compared with PCM control. The level of SGOT, SGPT & bilirubin significantly increased in Atractyloside group may be due to opening of mitochondrial permeability transition pore (MPTP). Treatment with EGCG (40mg/kg, p.o.) and Atractyloside (5mg/kg, p.o) showed hepatoprotection by significant reduction in serum SGOT, SGPT and bilirubin level in paracetamol treated animals when compared with Atractyloside and Paracetamol control rats shown in figure 1,2,3.

Effects of PCM and its combination on biochemical markers

Paracetamol produced a significant elevation in the TBARS when compared with untreated control rats. The level of TBARS significantly increased in Atractyloside group may be due to opening of mitochondrial permeability transition pore (MPTP). On the other hand, EGCG (40mg/kg, p.o) for 7 days with PCM treated rats showed significant

reduction in serum TBARS when compared with PCM control. Treatment with EGCG (40mg/kg p.o) and Atractyloside (5mg/kg p.o) showed hepatoprotection by significant reduction in serum TBARS level in paracetamol treated animals when compared with Atractyloside and Paracetamol control rats shown in figure 4.

Paracetamol produced a significant reduction in the SOD level in the present study when compared with untreated control rats. The level of SOD significantly decrease was observed in Atractyloside group may be due to the opening of mitochondrial permeability transition pore. On the other hand, EGCG (40mg/kg, p.o) for 7 days with PCM treated showed significant elevation in SOD level when compared with PCM control. Treatment with EGCG (40mg/kg p.o) and Atractyloside (5mg/kg p.o) showed hepatoprotection in term of significant elevation SOD level in Paracetamol treated animals when compared with Atractyloside and Paracetamol control shown in figure 5.

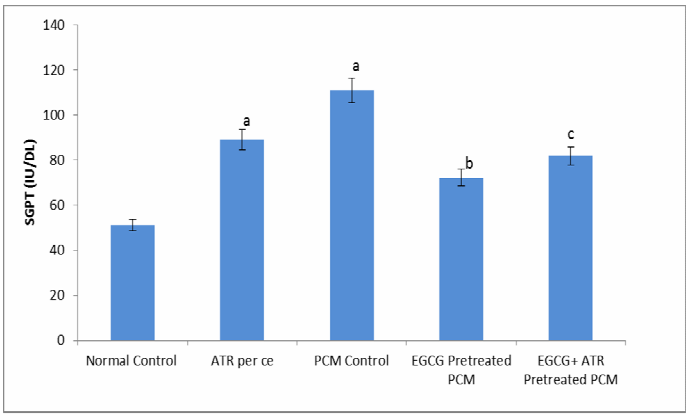

Fig. 1 : Effect of EGCG on SGPT level in PCM induced hepatotoxicity

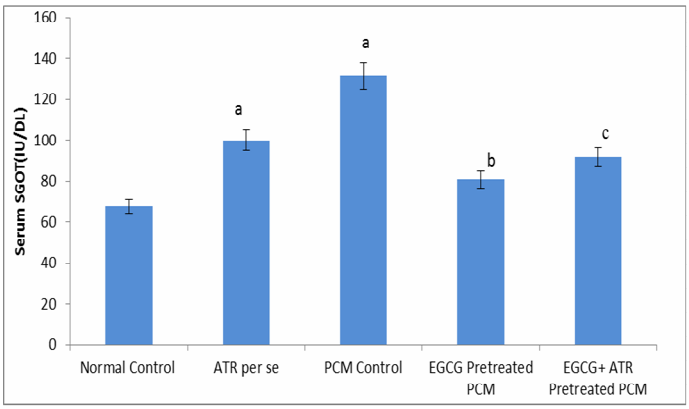

Fig. 2 : Effect of EGCG on SGOT level in PCM induced hepatotoxicity

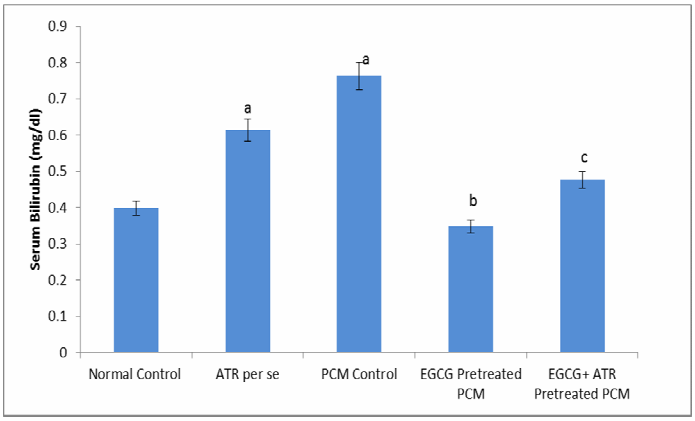

Fig. 3 : Effect of EGCG on Bilirubin level in PCM induced hepatotoxicity

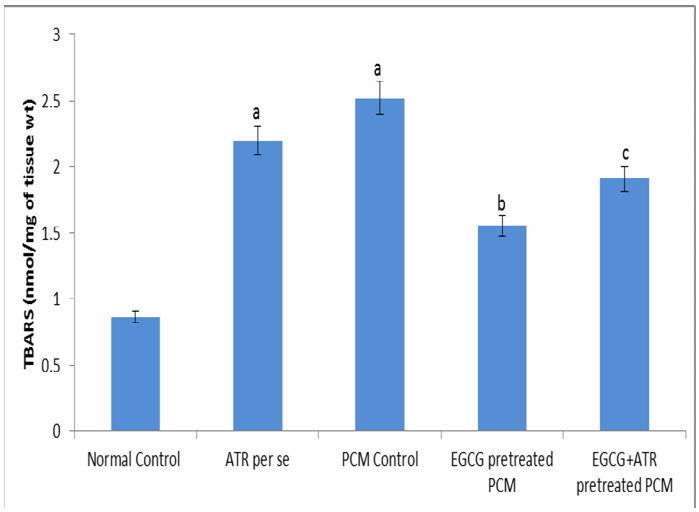

Fig. 4 : Effect of EGCG on TBARS level in PCM induced hepatotoxicity

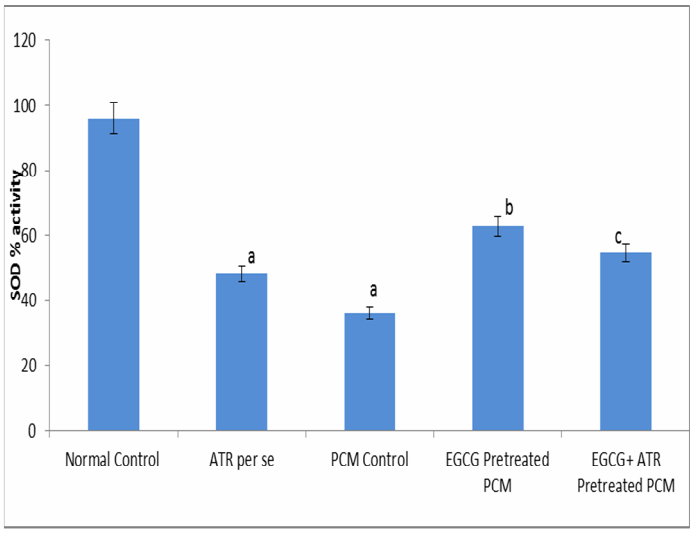

Fig. 5 : Effect of EGCG on SOD level in PCM induced hepatotoxicity

HISTOPATHOLOGICAL STUDY

Comparative microphotograph of liver sections from different group like, PCM (3g/kg), EGCG+ PCM (40 mg/kg), ATR control and PCM+EGCG +ATR treated groups of animals. Normal control group shown well defined hepatocytes around the central vein (Fig 6-A), PCM & ATR treated liver sections of treated wistar rats produced necrotic hepatocytes around the central vein (Fig 6-B,C). The reduced number of necrotic hepatocytes around central vein noted as near normal appearances of hepatocytes in PCM+ EGCG treated rats (Fig 6-D). The necrotic hepatocytes around central vein reduced as shown hepatoprotection in ATR+PCM+EGCG group as compare to ATR control group (Fig 6-E). Quantitative estimation of necrotic areas in control, PCM, ATR, PCM + EGCG, PCM + EGCG +ATR groups of animals noted. EGCG treatment significantly reduces the amount of necrotic areas around the central vein in PCM treated groups shown in figure 6.

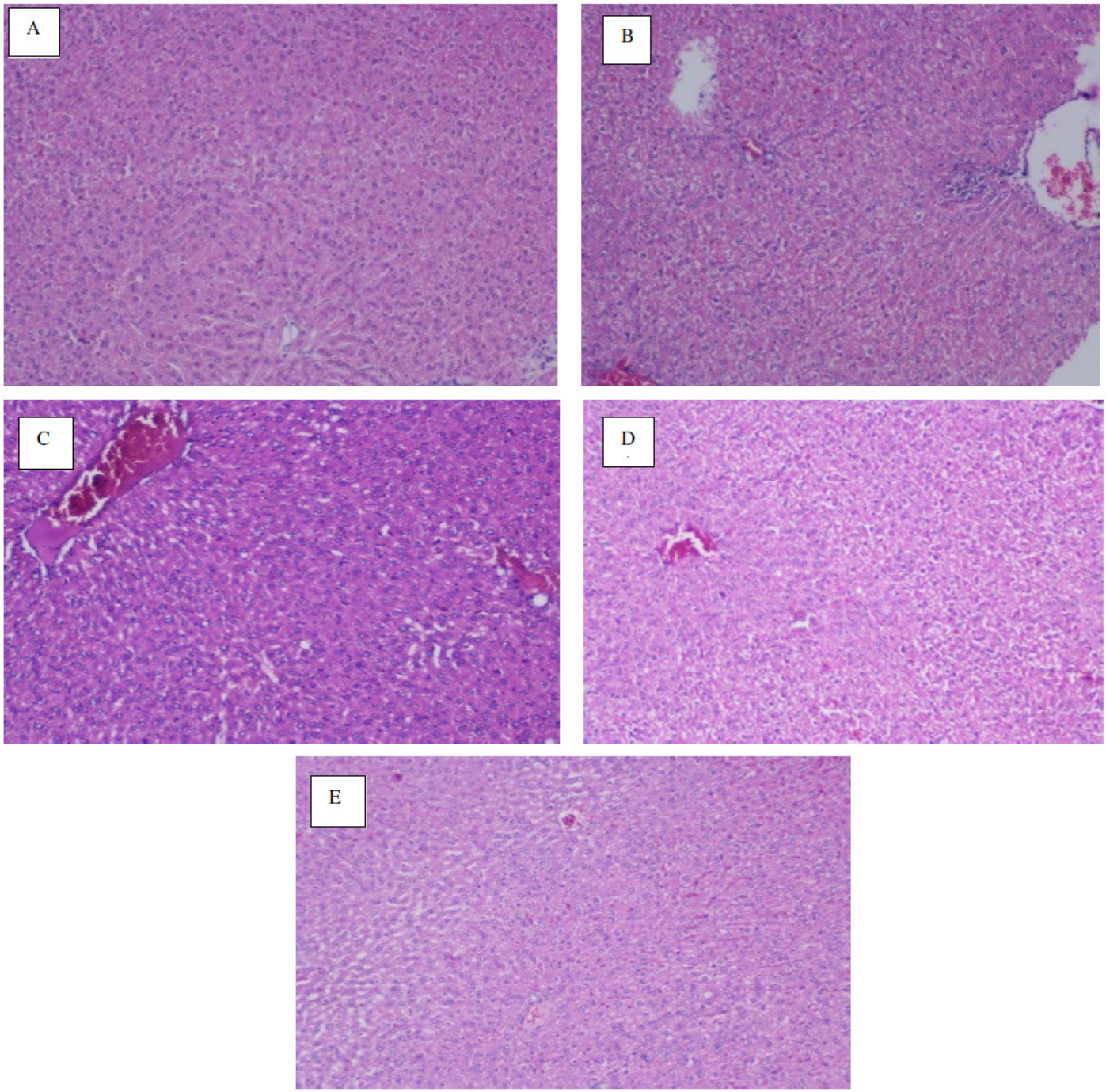

Fig. 6 : Effect of EGCG on Histopathological examination of Liver

Discussion

The finding of present study revealed that the EGCG has therapeutic potential to ameliorate paracetamol induced hepatic changes in rats.

Paracetamol is reported to produced hepatic injury at the dose 3g/kg p.o. in the well reported model for screening of hepatoprotective agents against drug induced liver injury (Tedeschi *et al.,* 2002). PCM forms glucuronide or sulfate on detoxification in liver. However, the overdose of PCM produces reactive metabolite NAPQI which further binds covalently to cellular macromolecules and initiates the cell damage (Huang *et al.,* 2014). It was due to oxidative stress followed by necrotic cell death induced by paracetamol induced hepatotoxicity (Ramadoss *et al.,* 2011). Moreover, oxidative stress is noted to induce mitochondrial dysfunction through activation of mitochondrial permeability transition pore (MPTP) and adenosine triphosphate (ATP) depletion in present study (Kaushal *et al.,* 1999). Paracetamol induced inflammatory mediators further activate hepatic stellate cells which consequently increase the expression of TGF, collagen and elastin resulting in cirrhosis of liver (Kessova and Cederbaum, 2007).

In the present study higher concentration of paracetamol 3g/kg body weight cause severe liver injury damage as observed increase level of SGOT, SGPT and bilirubin. Paracetamol at the dose 2g/kg and 3g/kg are reported to cause increase level of SGOT, SGPT and bilirubin in rodents (Jeong *et al.,* 2005).

On the other hand EGCG cause significant decreased the level of SGOT, SGPT and bilirubin. SGOT and SGPT are the metabolic enzymes and increase level of these enzymes significantly due to hepato-biliary duct damage by ROS generation, iNOS, COX-2, TNF-α, PI_3 Kinase, NF kappa B and decrease ATP. Therefore in the present study EGCG may have potential to block the ROS generation, iNOS, COX-2, TNF-α, PI_3 Kinase NF kappa B, increased ATP generation

leads to MPTP changes resulting increased level of SGOT, SGPT in blood upon injury. The Serum bilirubin level is another conventional indicator of hepatic cell secretory functions and its elevation is also attributed to hepatic insufficiencies in the Paracetamol induced liver dysfunction by ROS generation. However EGCG significantly blocks ROS generation (Shah *et al.,* 2010) expression of PPAR□, Inter leukines formation, TNF alpha, increased NO & ATP generation (Chen *et al.,* 2004). It has been observed that EGCG up regulate JAK/STAT, MAPK and PI_3K/AKT (Oz *et al.,* 2013) leading to hepatoprotection responsible for increase the above mechanism in serum upon PCM induced liver dysfunction.

Paracetamol on over dose cause liver injury via formation of NAPQ1 by cytochrome P4502E1 is reported to cause hepatotoxicity (Huang *et al.,* 2003; Jaeschke and Bajt 2006) and increase expression of iNOS, leading to oxidative stress and increase release of pro inflammatory mediators (IL-12, IL-18) upon activation of kupffer cell, TNF-α and hepatic steatic cell. These consequences lead to mitochondrial dysfunction which characterized by increase in Ca^{2+} and leads to ATP depletion. However in present study EGCG treatment showed significant decreased in the level of SGOT, SGPT, Bilirubin, TBARS and increased the level of SOD (Chacko *et al.,* 2010). Hence EGCG as potential to attenuated cellular events responsible for mitochondrial dysfunction and thereby preventing MPTP opening. This content is reported by earlier study mentioning that EGCG at the dose of 40mg/kg decrease oxidative stress, i.e. TBARS and SOD in rats.

The protective effects of EGCG in ameliorating PCM induced hepatic changes by preventing the MPTP opening is confirmed by the treatment of Atractyloside (selective MPTP opener) since atractyloside alone is unable to cause severe hepatic injury but only caused inflammation as in the form of significant increase in serum bio chemicals and tissue bio chemicals as compared to normal control. These results are found to be significantly low as compare to paracetamol control.

Conclusion

The present study is designed to investigate the beneficial effects of EGCG on PCM induced liver injury in rats and to find out the involvement of mitochondrial permeability transition pore, oxidative stress marker, SGOT, SGPT, Bilirubin and inflammatory pathway in liver injury. Rat was administered PCM (3g/kg/day, *p.o.*) on 3rd and 5th days of 7 days protocol to produce hepatotoxicity. The index of liver damage was assessed by measuring the level of SGOT, SGPT and bilirubin. Moreover, the oxidative stress in liver was assessed by measuring TBARS and SOD. The result of present study suggested that EGCG both in preventative and curative regimen significantly attenuated liver dysfunction and oxidative stress induced by PCM and Atractyloside. In the present study paracetamol treatment caused severe fatty change vacuolization, cellular degeneration extension of cellular vein and necrosis with nuclear pycnosis in hepatic cells. However EGCG 40mg/kg significantly restored normal architecture of liver and ameliorated PCM induced histopathological changes and this protection was found to be attenuated on treatment with Atractyloside. The finding may be concluded in the present study that paracetamol causes severe liver damage which is characterized by increased oxidative stress and mitochondrial dysfunction due to opening of MPTP. However EGCG is a potent anti-oxidant prevented the opening of MPTP and thereby arrested mitochondrial dysfunction upon paracetamol exposure. Moreover, further studies for measuring the expression of MPTP and calcium ions concentration during hepatotoxicity may be warranted.

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