



## Hepatoprotective effects of arabica coffee beans in paracetamol induced hepatotoxic animal models

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### Abstract

Drug detoxification functioning of liver exposes it to a variety of toxic metabolites and the damage due to toxins can lead to liver diseases. However, treatment options for liver pathologies are very limited in conventional medicines, therefore, the focus has been shifted more towards alternatives routes to restore the functions of liver. Coffee is a widely consumed beverage that has exhibited improvement in liver physiology. This study was carried out to investigate the hepatoprotective effects of Arabica coffee beans by in New Zealand rabbits that exhibited drug toxicity following an overdose of paracetamol via oral ingestion. To evaluate whether coffee offers hepatoprotection at earliest hour of its consumption, a group of animals received co-treatment of Arabica coffee beans and paracetamol. Another group received paracetamol only. A control group of animals was also included for comparison. Liver function, lipid profile, renal efficiency and CYP2E1 gene expression of all the animals in each group were estimated. Arabica coffee beans showed a decrease in ALT and ALP levels and restoration of ferritin. Lipid profile tests displayed that coffee group showed a reduction in TL and TC level, TG were elevated and LDL were restored and no change was found for HDL levels. Coffee consumption was found to increase the urea and creatinine levels. Upregulated gene expression of CYP2E1 indicated liver injury in paracetamol group whereas coffee significantly downregulated it. Thus, coffee beans exhibited hepatoprotective actions along with the restoration of lipid profile in acute liver injury animal models of 4 hour.

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## Introduction

Liver, being an essential body organ performs a variety of functions. The total number of functions performed by liver is estimated to be greater than 500 (Davidson and Trey, 2007). Since liver is involved in essential bodily functions, therefore diseases of liver are a major concern worldwide (Adewusi and Afolayan, 2010). The term hepatic disease represents a cellular, structural or functional damage to the liver, thus conditions like excessive alcohol consumption, autoimmunity, pathogens and drugs can inflict liver damage (Davidson and Trey, 2007). Much focus has been exerted towards the role of liver in detoxification of exogenous and endogenous waste (Adewusi and Afolayan, 2010). However, out of all factors that contribute in hepatic diseases, liver injury due to adverse effects of drugs is considered as a most prominent cause.

The term liver injury incorporates both, an insult to liver directly by a drug or by any of its toxic metabolites (Pham *et al.*, 1997). Many drugs are responsible for the acute liver failure (Russo *et al.*, 2004). Upon drug induced liver injury, utilization of harmful drug is often discontinued, whereas patients might need to sort towards short term hospitalization or liver transplantation (Smith and Schmid, 2006). Drugs can cause both predictable and unpredictable liver injury which may represent either, a high or a low incidence rate respectively. For instance, paracetamol (PCM) or acetaminophen, an over the counter antipyretic and analgesic may give rise to predictable liver injury within a few days (Pham *et al.*, 1997). It is also well known that approximately 50% cases of acute liver failure initially develop from drug induced liver injury (Kaplowitz, 2001; McNally, 2010). Thus, there is a continuous search all around the world for treatments which could restore liver function (Mushtaq *et al.*, 2014).

In modern medicine, there are no effective cures for protection of liver or stimulation of a liver function (Bhawna and Kumar, 2009). However, many medicinal agents of high value can be found within plant kingdom (Mushtaq *et al.*, 2014), therefore

researches are trying to find the remedies of liver disease from the natural plant products. Coffee is one such plant source which shows positive effects on liver health. Coffea plants have berries and the seeds of these berries are widely consumed as the famous beverage, coffee (Maurin *et al.*, 2007). Coffee is consumed for its stimulating effects and good taste, in addition to this, it also provides protective effects against liver diseases such as chronic liver disease (Ruhl and Everhart, 2005; Setiawan *et al.*, 2015), hepatocellular carcinoma (Setiawan *et al.*, 2015), cirrhosis (Liu *et al.*, 2015), hepatitis C virus (Batista *et al.*, 2014) and non-alcoholic fatty liver disease (Molloy *et al.*, 2011).

The current study aimed at identifying whether Arabica coffee beans confer hepatoprotective actions on acute liver injury resulting from toxic oral overdose of PCM in a smaller 4 h time duration setting.

## Material and methods

### Coffee preparation

Roasted Arabica coffee beans (DAVIDOFF CAFÉ, Colombia) weighing 30 g were boiled in 1 L of distilled water for 2-3 min, later cooled, filtered and stored at - 20 °C till future use (Alshammari *et al.*, 2017). There was no addition of any other material.

### Experimental animals

Healthy male and female New Zealand rabbits were bought in equal sex ratio from a local market in Karachi, Pakistan. The animals weighed between 0.5 kg to 1.8 kg. The rabbits were acclimatized to the laboratory conditions where they were kept in cages, fed with normal chow and allowed free access to water.

### Treatment groups

The study was designed as a cross sectional, randomized controlled trial and liver injury was induced in animals by giving an over-dosage of PCM (Panadol 500 mg tablet, GlaxoSmithKline, Karachi, Pakistan) orally (Sabina *et al.*, 2011). Three groups of the animals with at least five animals in each group

received following treatment;

**Control group:** Control animals received normal feed and water.

**PCM group:** PCM at a dose of 1g/kg of body weight/animal.

**Coffee group:** Coffee 5 mL concoction in addition to PCM at a dose of 1g/kg of the body weight/animal.

#### *Blood collection*

Animals were sacrificed to collect the blood samples in aseptic conditions. Serum separation was carried out through centrifugation and the obtained serum was stored at -20 °C until further use.

#### *Biochemical studies*

ALT and ALP were used to estimate the liver function (Zilva *et al.*, 1975; Burtis *et al.*, 2012). Ferritin estimation was carried out to test iron storage capacity (Burtis *et al.*, 2012).

High-density lipoproteins (HDL), Low-density lipoprotein (LDL), total cholesterol (TC) and triglycerides (TG) estimation were performed to evaluate lipid profiling (Burtis *et al.*, 2012). Renal efficiency was assessed by determining the levels of urea and creatinine (Talke and Schubert, 1965).

#### *RNA isolation*

RNA isolation was achieved using a kit (Trizol, Sigma-Aldrich, Life Sciences Technologies) reagent as per direction of the manufacturer. Serum sample was incubated for 5 minutes at room temperature after adding RNA extraction reagent. After adding chloroform, the cell pellet was washed, centrifuged, air-dried and re-suspended in RNase free H<sub>2</sub>O. Using spectrophotometer the yield and purity of RNA fraction was quantified and the samples were stored at -70 °C.

#### *Complementary DNA synthesis*

For the transcription of mRNA, using the kit (QuantiTect Reverse Transcription kit, Qiagen) and following the instructions of manufacturer, 500 ng of RNA was transcribed in to a first-strand

complementary DNA (cDNA). Later, reverse transcription of RNA samples with random Oligo dT was performed in a single cycle at 65 °C for 5 minutes in a PCR thermal cycler (Applied Biosystems Corp.) as per manufacturer's instructions. The samples were diluted in RNase free H<sub>2</sub>O and stored at -4 °C till further use.

#### *Quantitative Polymerase Chain Reaction (qPCR)*

For gene expression analysis qPCR was performed, in which the primers (Eurofins Genomics, USA) were combined with the cDNA. For attaining final 1X concentration in a reaction mixture, master mix (QuantiTect Sybr green PCR kit, Qiagen) was inserted in the reaction tube. Temperature conditions for this study were maintained to be within the linear range related to real-time input and PCR cycles. PCR cycles were executed as follows; initialization was done at 50 °C for 2 minutes, and later denaturation, annealing and extension were performed at 95 °C, 60 °C and 72 °C for 15, 30 and 30 seconds respectively in a 7300 PCR system (Applied Biosystems Corp.). The calibration of data was with relevance to the untreated control's expression. Expression change was analyzed via fold change using GraphPad prism (Table 1).

#### *Statistical analysis*

Bar graphs are used to present the data generated through the experiment. Each column represent mean value and error bars are represented by  $\pm$  standard deviation to express the data. A one-factor ANOVA was used to compare variances within each group followed by post-hoc Tukey's test. The P value of < 0.05 was considered statistically significant.

#### **Results**

The liver functioning tests showed a markedly higher level of ALT (Fig. 1a) in PCM group as compared to the control, whereas ALT level upon coffee+PCM co-treatment showed a statistically significant reduction. We found that changes in the ALT level among all groups were not drastic. The drug overdose animals in PCM group showed a slight increase in ALP level (Fig. 1b) as compare to the coffee+PCM and control

group of animals. Theserum ferritin assessment (Fig. 1c) showed that both, the control and coffee+PCM group has similar level of ferritin while the PCM group showed moderately reduced levels of it.

In serum lipid profiling(Fig. 2a), as compare to control group, the TL level in PCM was lower, and these levels reduced even further in coffee+PCM co-treated group. HDL, considered as good cholesterol was found to be not much affected in our study (Fig. 2b). None-the-less, HDL level was found slightly raised in coffee+PCM group, whereas the control and PCM group demonstrated similar levels of HDL. On the other hand, LDL isdepicted as bad cholesterol, the

levels of which werealso not much affected. After 4 hours of PCM consumption, LDL was slightly raised in PCM group but the coffee+PCM treatment demonstrated that it reached back to the level of control group (Fig. 2c). We found that PCM group showed a slightly reduced TC level (Fig. 2d), but upon co-treatment coffee+PCM group showed a moderate reduction in TC as compare to control group. High levels of TG are associated with improper metabolism. In our study, we found that PCM has reduced the TG within few hours of oral consumption, however coffee+PCM group indicated an even more pronounced reduction in TG level, when compared to control group (Fig. 2e).

**Table 1.** Gene primer used in qPCR for gene expression analysis of CYP2E1 in an acute liver injury model.

Gene name	Gene symbol	Oligomer sequence (5' to 3')	Length (bp)
Cytochrome P450 2E1 (Peng and Coon, 1998)	CYP2E1-forward	CATCGGGAATCTTCTCCAGTTGG	23
	CYP2E1-reverse	TGAAGGGTGTGCAGCCGATGACAA	24
Glyceraldehyde 3-phosphate dehydrogenase (Accession number NM_001082253.1)	GAPDH- forward	AGTAAGAGCCCTCAAACCACC	21
	GAPDH- reverse	TGGGATGGAAACTGTGAAGAGG	22

For the estimation of kidney functioning, urea and creatinine assessment was performed. Urea level (Fig. 3a) in PCM group was almost samewith that of control group but it elevated failry more in PCM+coffee co-treated group. Contrastingly, creatinine level (Fig. 3c) was found to be similar incoffee+PCM group and control group but it was considerably lower in PCM group.

Relative gene expression of CYP2E1 showed an upregulation of CYP2E1 gene (Fig. 4) in PCM treated group wherase, the statistically significant downregulation was evident in PCM+coffee co treated group, when compared with control.

## Discussion

Our study showed that PCM overdose increased the levels of acute liver injury marker and coffee resisted this effect. In case of ALT, the effects were more conspicuous and the reduction of ALT levels was marked, indicating that coffee resisted the liver deterioration change of PCM and improved the liver

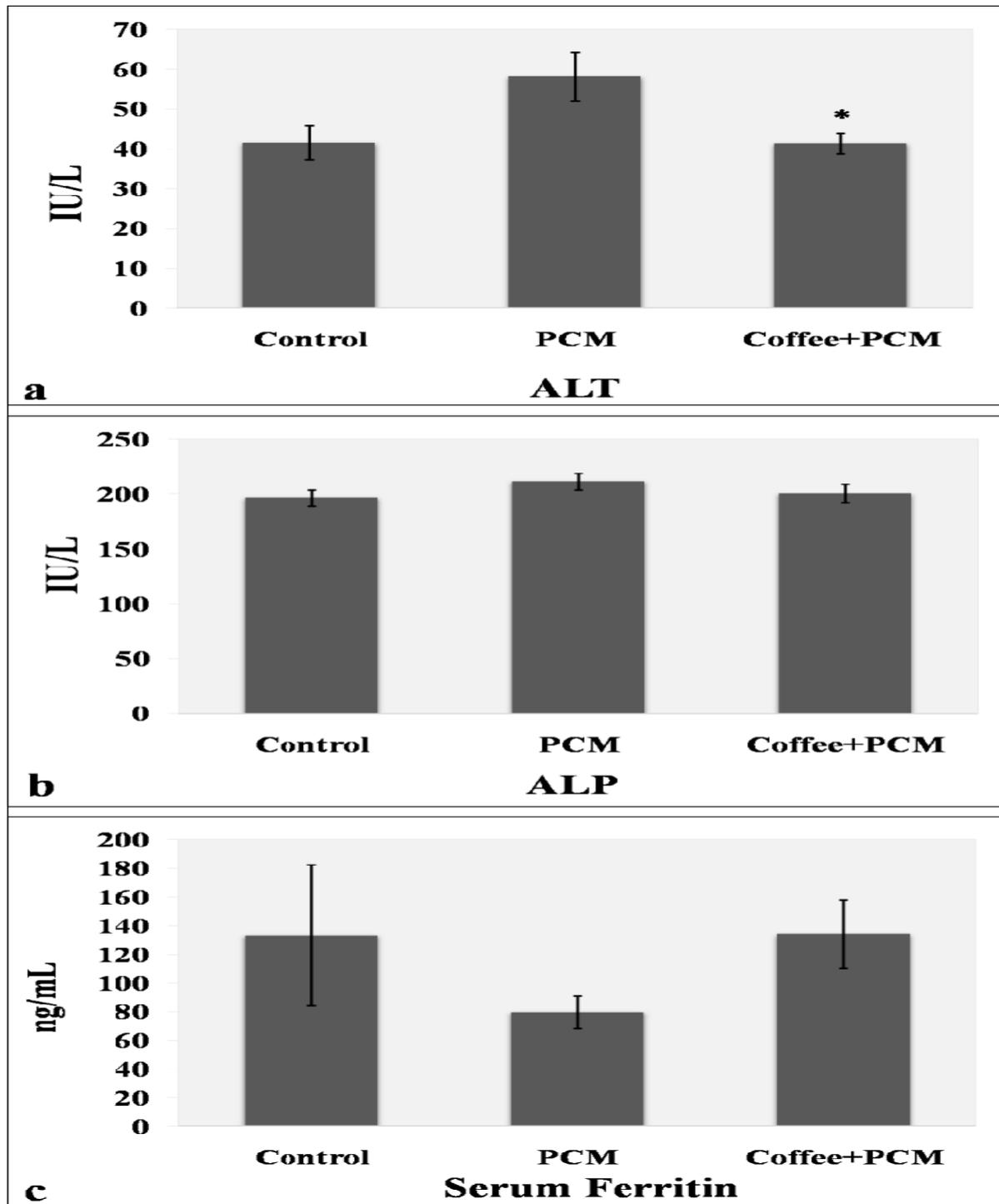
functioning. It is well established that ALT and ALP enzymes are effective markers to identify the type and extent of liver damage as these get released from cytosol of hepatocytes into the blood circulation upon an insult to live round that administration of coffee effectively prevented the acute liver injury by PCM, thus the regular consumption of coffee may also prove to become beneficial for improving liver function.

These results are in accordance to other studies in which coffee consumption displayed reduction of liver functioning enzymes such as, AST, ALT, ALP and GGT ell as prevention from cirrhosis and fibrosis Ferritin is another marker for identifying liver health status as it raises upon acute liver damage and is reported as a more sensitive measure in comparison to transaminase based liver functioning enzymes (Eastham *et al.*, 1976).

Interestingly, in our study, serum ferritin was found to be increased upon coffee and PCM co-treatment

but these reached back to a similar level as that of control group. Even though, an association between high serum ferritin and acute liver failure is established (Anastasiou *et al.*, 2017), we could not find any correlation between liver injury enzyme

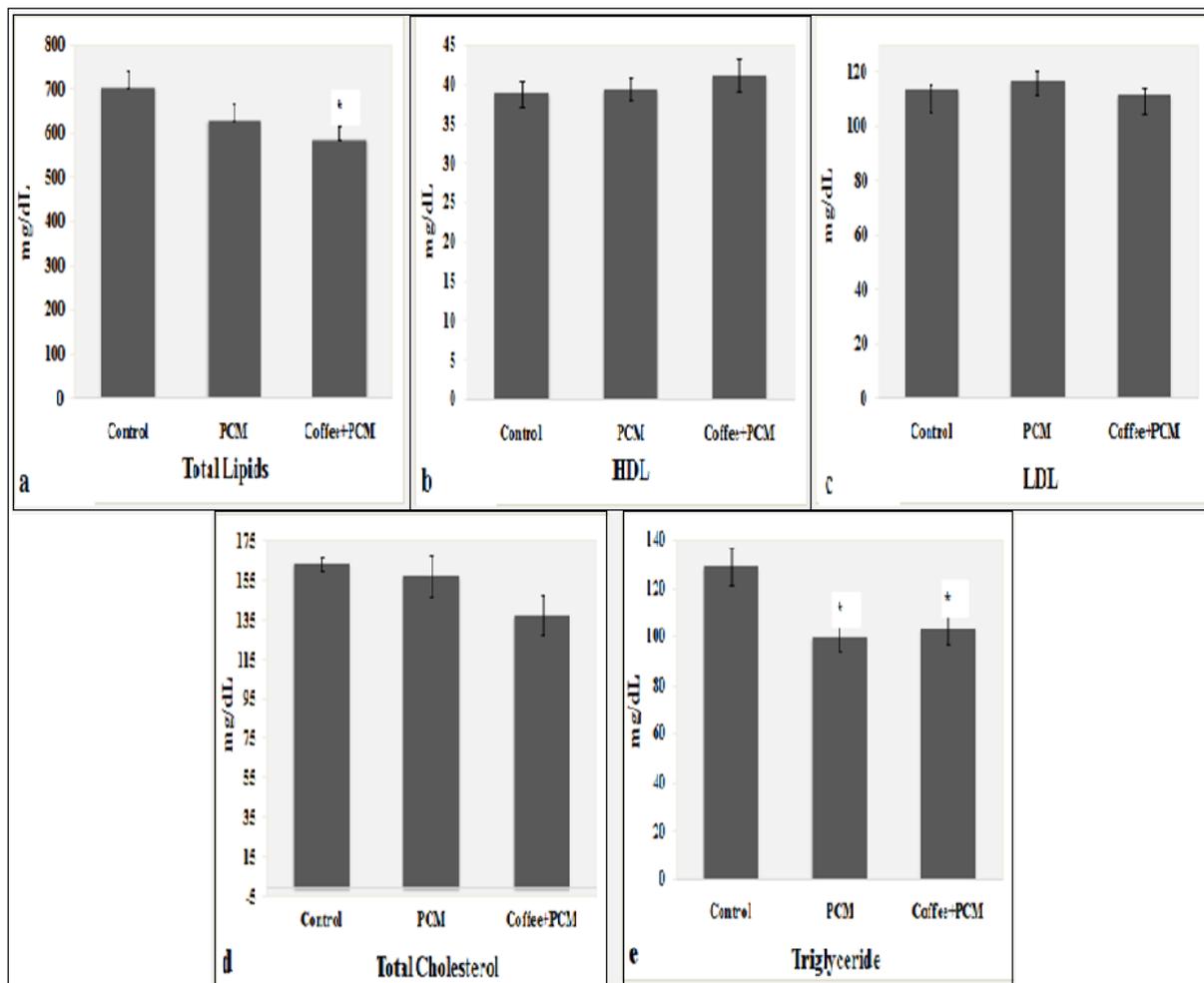
markers and ferritin content. Thus, coffee most probably exerted no beneficial nor any harmful effect on serum ferritin when PCM induced acute liver injury has occurred at 4 hours' time period.



**Fig. 1.** Estimated levels of (a) ALT (b) ALP and (c) ferritin in control, PCM, and coffee + PCM in animal model. Each column represents the mean value while each error bar represents the  $\pm$  standard deviation. \*shows results which were found statistically significant  $P < 0.05$  following post hoc Tukey's test as compared to PCM group.

Since liver is the major site for lipid metabolism, estimation of lipid profile estimation is an important factor in the determination of the liver physiology. Interestingly, coffee consumption was found to affect lipid profile positively even after a mere ingestion period of 4 hours. We found that PCM decreased TL whereas, addition of coffee aggravated this reduction

even further. Although, PCM did not affect lipoproteins much, but coffee slightly increased the HDL and reduced the LDL. PCM displayed a decrease in TC and coffee co-treatment with PCM boosted this effect. We found that both, PCM and coffee co-treatment with PCM markedly decreased the TG.



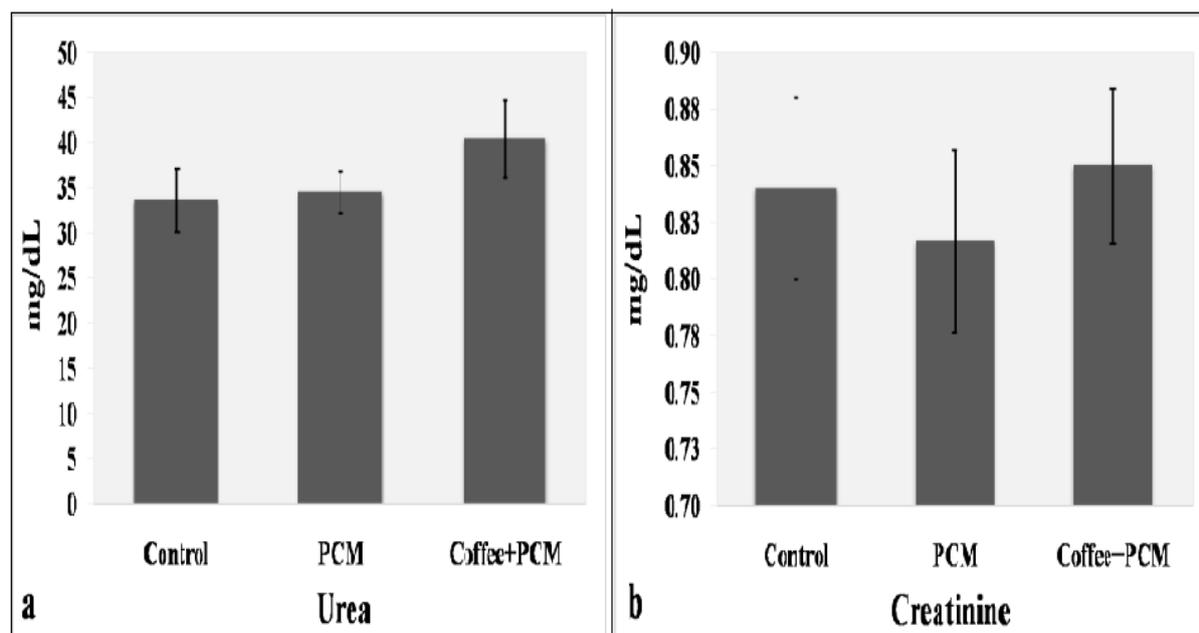
**Fig. 2.** Lipid profile assesment includes (a) TL, (b) HDL (c) LDL, (d) TC and (e) TG levels obtained in control, PCM and coffee + PCM groups. Each column represents the mean value while each error bar represents the  $\pm$  standard deviation. \* shows that results were statistically significant, where  $P < 0.05$  represents statistical significance.

In our study, coffee decreased the levels of TG, TC and LDL in co-treated group, while an elevation in HDL levels is also there. This is contradictory to other studies which showed that coffee consumption increase the levels of TG, TC and LDL (Aro *et al.*, 1990; Cai *et al.*, 2012). However, not much information is available about the mechanism through which coffee affects lipid profile. But it is an

established fact that decreased total lipids, total cholesterol and LDL are considered bad for health while increased the HDL which is good cholesterol (Toth, 2005). The results of our study differ might be due to the acute nature of the injury that span only a few hours post drug overdose. This could also be attributed to the various bioactive molecules identified (Urgert *et al.*, 1997), along with various fat-

soluble compounds (Lee, 2000). It is suggested that, both, the type and mode of coffee preparation strongly affect the lipid profile modulation (Corrêa *et al.*, 2013). The bioactive molecules, cafestol and kahewol are found in considerable amounts in unfiltered boiled coffee (Ratnayake *et al.*, 1993; Weusten-Van der Wouw *et al.*, 1994; Urgert *et al.*, 1997), that enhance TG, TC and LDL (Heckers *et*

*al.*, 1994; Weusten-Van der Wouw *et al.*, 1994; Urgert *et al.*, 1997). One reason might be the preparation method of choice, as we used roasted coffee beans which were boiled and filtered, this could have affected the concentration (Thelle *et al.*, 1983; Ratnayake *et al.*, 1993) of active components in our preparation.

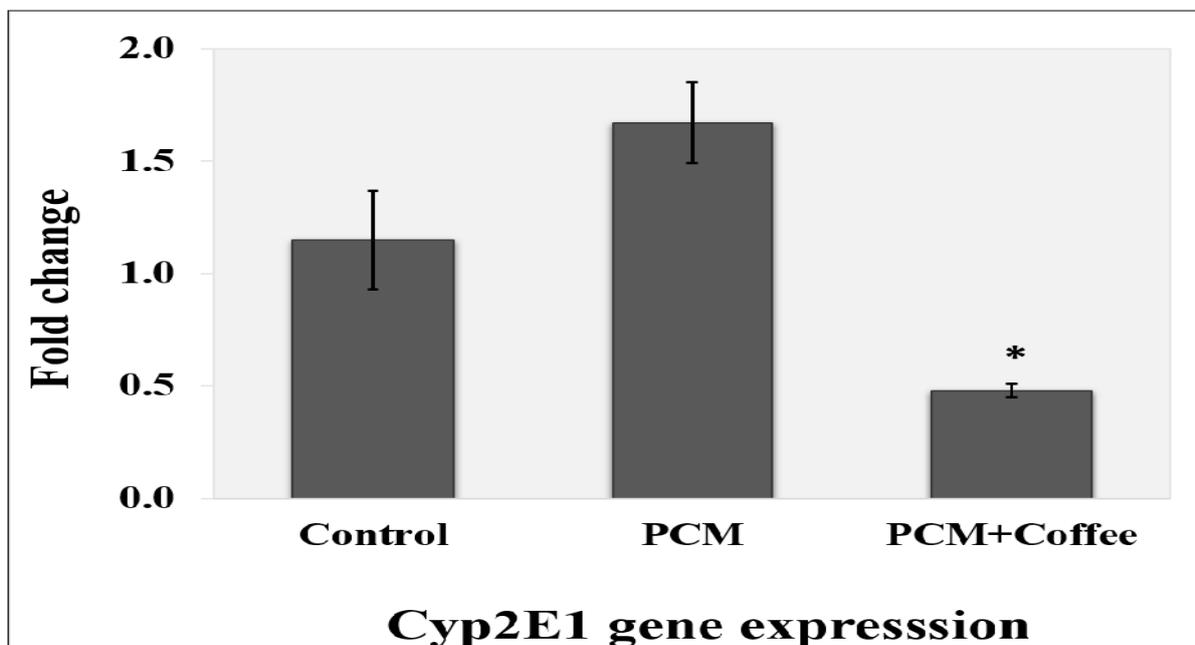


**Fig. 3.** Evaluation of coffee consumption in renal efficacy via (a) urea and (b) creatinine estimation in control, PCM and coffee + PCM groups. Each column represents the mean value while each error bar represents the  $\pm$  standard deviation. \* denotes that results were statistically significant, where  $P < 0.05$  represents statistical significance. † shows results were statistically significant  $P < 0.05$  following post hoc Tukey's test as compared to PCM group.

Hepatic and renal failures are strongly relatable (Kher and Makker, 1987). Thus, in order to evaluate the efficiency of liver injury, estimation of renal functioning parameters can not be neglected. PCM overdose is known for inducing harmful effects on renal function that may also lead to renal failure (Kolawole *et al.*, 2014). We found that PCM did not have pronounced effects on increasing the urea level but coffee and PCM together increased the urea levels. Liver plays a role in urea cycle and converts the ammonia into urea which later eliminates via urine (Kolawole *et al.*, 2014), excessive level of urea indicates deteriorating renal functions (Pedraza-Chaverri *et al.*, 2004; Yang *et al.*, 2012). In contrast, PCM group showed a reduction in the creatinine level

but coffee and PCM co-treatment elevated it to the level similar to that of control group. Creatinine is a by-product of muscle metabolism, and it increases in serum when there is impaired renal filtration (Kolawole *et al.*, 2014).

However, it can be assumed that this result does not indicate that coffee administration negatively impacted renal function because both, the lipid profile and hepatic functions were found to be improved. This increase in urea and creatinine could be due to caffeine, which is a main coffee component and is often associated with these mentioned biochemical phenomena (Pashmforoosh *et al.*, 2015; Emmanuel *et al.*, 2017).



**Fig. 4.** Effect of coffee consumption on CYP2E1 in a 4 h acute liver injury animal model. Each column represents the mean value while each error bar represents the  $\pm$  standard deviation. \* shows that results were statistically significant, where  $P < 0.05$  represents statistical significance. † shows results were statistically significant  $P < 0.05$  following post hoc Tukey's test as compared to PCM group.

In order to strengthen our study, we performed qPCR for the CYP2E1 relative gene expression. Our data displays that 4 hours following PCM ingestion induces upregulation of CYP2E1 gene expression indicating liver injury whereas coffee group successfully downregulated. CYP2E1 belongs to the family of cytochrome P450 enzyme proteins, that breakdown the drugs and synthesis of lipids.

As coffee treatment showed improvement in ALT and lipid profile, we did anticipate that CYP2E1 expression will be reduced. This could be associated with toxic PCM dose, that possibly lead to interaction between CYP2E1, NAPQI and drug metabolites hence influencing the reduction in CYP2E1 (Snawder *et al.*, 1994; Sinclair *et al.*, 2000). We therefore suggest that Arabica coffee beans induce a hepatoprotective effect and can fight the drug induced liver injury actions of PCM.

### Conclusion

Drug induced liver injury is associated with complications such as dyslipidemia and renal function compromise. We found that coffee consumption resists the PCM induced acute liver

injury potential and begin offering hepatoprotection within few hours of its oral ingestion. Since coffee reduced the levels of liver functioning enzyme, ALT in PCM induced acute liver injury thus, it prevented the adverse effects of PCM toxicity. Coffee also improved the lipid profile parameters. Considering that CYP2E1 is also associated with the drug metabolism and lipid synthesis, the down regulation of CYP2E1 gene expression in co-treated group indicated that coffee promoted the hepatoprotective actions. Further studies are needed to identify the mechanism behind the protective actions of coffee, nonetheless findings of this study are expected to contribute towards options to fathom acute liver injury.

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### Conflict of interest

There is no conflict of interest in this study.

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