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Protective role of coffee supplementation in liver cirrhosis: Study in rats

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Abstract: Present study was designed to evaluate the effects of coffee on liver function tests and liver antioxidant enzymes in thioacetamide induced liver cirrhosis in rats. Experimental study period was consisted of eighteen weeks divided into two phases. Therefore 24 rats were distributed randomly into four groups (n=6). Group I served as control. In phase I, group II and III received thioacetamide (200mg/kg body weight intraperitoneally twice a week) and group IV received saline for 12 weeks. In phase II, group II received saline while group III and IV received an oral dose of coffee (0.4mg/Kg b.w) daily for 6 weeks. At the end of the study period rats were sacrificed and blood was collected to get serum and liver was homogenized for the determination of antioxidant enzymes. Marked increase in serum total and direct bilirubin, ALT, AST whereas reduced ALP was observed in test group. The reduced tissue SOD activity and increased tissue catalase and tissue MDA activity were also observed in test group. However, coffee consumption in group III in phase II significantly restored liver biomarkers and the tissue antioxidant enzymes SOD, catalase and MDA activities. In conclusion, thioacetamide induced liver cirrhosis can be prevented by coffee supplementation.

Keywords: Coffee, thioacetamide, liver cirrhosis, liver function tests, antioxidant enzymes.

INTRODUCTION

In Human culture, coffee had a huge space since 1200 years. According to American Food and Drug administration coffee has a strong protective status, noticing a caffeine substance of coffee commonly identified as safe with no health risk found. Coffee is popular due to its typical pleasant fragrance, taste and caffeine which considered as an additional part of it. In resent, a thought is proposed on important impact of coffee drinking in reducing total and reason specified mortalities. Coffee is comprised of many different compounds such as carbohydrates, lipids, vitamins, nitrogenous compounds, antioxidants, phenolic compounds, alkaloids etc. According to Higdon *et al* coffee utilization lessened several chronic disorders and its protective effects also ameliorate disease conditions such as cardiac disease, stroke, diabetes type II and also Parkinson's disease (Freedman *et al*., 2012; Lopez-Garcia *et al*., 2008; Huxley *et al*., 2009; Wu *et al*., 2009; Ascherio *et al*., 2001; Farah *et al*., 2012; Higdon *et al*., 2006; Heckman *et al*., 2010).

Many studies on positive effects of coffee consumption on liver markers and hepatopathies have been gathered about last twenty years. Consuming coffee has noticed an inverse relationship with liver markers (AST, ALT, ALP and GGT). Increased usage of coffee (more than 2 cups/day) related with reduced risk of increased ALT and also reduced the risk of hepatopathies (Arnesen *et al*., 1986; Mod *et al*., 2010). Coffee was described as activator of number of enzymes take part in liver detox pathway inclusive of Uridine 5'-diphospho-glucuronosyl transferases (UGT), the earlier research described that Diterpines were accountable for the UGT activation. The Kalthoff *et al* moreover described, the UGT activation through coffee did not depend on caffeine whereas the main Diterpines in coffee are cafestol and kahweol. Moreover editing in this study, the Gressner described that coffee as well as caffeine both activate several liver preventive molecular mechanism of signaling and they are still under investigation in respect of coffee beneficial effects (Huber *et al*., 2002; Kalthoff *et al*., 2010; Gressner, 2010). The fibrosis diminished characteristics also described by coffee in animal model where thioacetamide induced hepatic damage and coffee administration showed reduced necroinflammation and fibrosis (Furtado *et al*., 2012). The Japanese study proposed coffee consumption showed liver protective effects through inverse relationship with leptin, high sensitivity C-reactive protein and liver markers (Yamashita *et al*., 2012).

Cirrhosis in developed countries is a big reason of morbidity and death. It is the final stage of hepatic fibrosis which is associated with liver vascular deterioration. It ends up in collapsing arterial and portal blood circulation right into the central vein (hepatic outflow), interfering exchange among liver sinusoids and hepatocytes. The fenestrated endothelial cells are lined on liver sinusoids which lie on the layer of connective tissues called the space of disse (perisinusoidal space) which comprised of few mono nuclear cells and hepatic stellate cells (HSC). In cirrhosis, the space of Disse is occupied by scar tissues and the fenestrations of endothelial cells are lost, the process called sinusoidal capillarization, it resultantly caused liver abnormal synthetic function.

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Thioacetamide, a white crystal like organosulfur compound causing hepatic deterioration and carcinoma activity (Ichimura *et al*., 2010). Thioacetamide administration metabolically converted into Thioacetamide-S-oxide and Acetamide instantly. Thioacetamide-S-oxide bound with cell macromolecules which are accountable for the alteration in permeability and calcium absorption in cells. This interference in calcium reservoirs enhances volume of nucleus, extend nucleoli, decrease mitochondrial activity ultimately cause liver necrosis (Hajovsky *et al*., 2012; Bruck *et al*., 2004). Thioacetamide caused large production of Reactive oxidative species which may overtake the defensive activity of antioxidants. Hence damages cell components like Protein, lipid, DNA and consequently affects structure and function of cell (Ansil *et al*., 2011).Thus, present study is designed to evaluate antioxidant property of coffee in thioacetamide induced liver cirrhosis.

MATERIALS AND METHODS

Twenty fours male Spragrue Dawley rats (200-250grams body weight) were purchased from the animal house of the Agha Khan University Hospital Karachi, Pakistan. They were caged separately in temperature maintained room 24±3ºC. The rats had free access to water and standard diet throughout the experiment.

Ethical guideline

The conducted study followed the ethical lines provided by Ethical Review Board as well as worldwide approved guideline for Animal care in research and Laboratory use (Health research extension Act of 1985). Experimental work was carried out in clinical biochemistry and hematology research lab, in department of biochemistry, Federal Urdu University, Karachi approved by Ethical Review Committee (No.2003).

Study design

The rats were randomly divided into four groups, each of six rats. The duration of the study was 18 weeks, divided into two phases. Thioacetamide (TAA) and Coffee were administered in either phase.

Group I: the control (remained untreated).
Group II: Test (TAA treated)
Group III: Test + Supp (TAA+ Coffee treated)
Group IV: Supplement (Coffee treated)

In Phase I, Test group and Test+Supplement group received TAA, dissolved in 0.9% NaCl, intraperitoneally at a dosage of 200mg/kg b.w, twice a week for 12 weeks. Supplement group only received coffee (orally at a dosage of 0.4mg/kg b.w, daily for 6 weeks and then received saline). In phase II, Test group received saline whereas Test+Supplement group received Coffee (orally at a dosage of 0.4mg/kg b.w daily for 6weeks) after TAA in first phase to study the hepatoprotective role of Coffee. At the end of experimental period, rats of all groups were decapitated. The blood was collected from the neck wound in tubes and centrifuged to collect serum. Liver was excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination, dried by blotting with filter paper and weighed. The tissues then kept in freezer at -70ºC until analysis.

Assessment of serum total and direct bilirubin and liver specific enzymes

Serum total and direct bilirubin (Sherlock, 1951), serum ALT ((Retiman and Franhel, 1957), serum AST, serum ALP were analyzed by using commercially prepared reagent kits.

Assessment of antioxidant status

Preparation of Post mitochondrial supernatant (PMS)

Liver homogenate was prepared by taking 1gm of liver tissue in 10ml of 5mM potassium phosphate buffer (pH 7.8) by using a homogenizer.

The homogenates were centrifuged at 800g for five minutes at 4ºC to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500g for 20 minutes at 4ºC to get post mitochondrial supernatant which was used to assay tissue superoxide dismutase, Catalase and MDA activity.

Estimation of lipidperoxidation

The malonyldialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thio-barbituric acid reacting substances (TBARS). Briefly, the reaction mixture consisted of 0.2ml of 8.1% sodium dodecylesulphate, 1.5ml of 20% acetic acid solution adjusted to pH 3.5 with sodium hydroxide and 1.5ml of 0.8% aqueous solution of thiobarbituric acid was added to 0.2ml of 10% (w/v) of PMS. The mixture was brought up to 4.0ml with distilled water and heated at 95°C for 60 minutes. After cooling with tap water, 1.0ml distilled water and 5.0ml of the mixture of n-butanol and pyridine (15:1v/v) was added and centrifuged. The organic layer was taken out and its absorbance was measured at 532nm and compared with those obtained from MDA standards. The concentration values were calculated from absorption measurements as standard absorption.

Estimation of tissue catalase enzyme

Catalase activity was assayed by the previously described method. Briefly, the assay mixture consisted of 1.96ml phosphate buffer (0.01M, pH 7.0), 1.0ml hydrogen peroxide (0.2M) and 0.04ml PMS (10%) in a final volume of 3.0ml. 2ml dichromate acetic acid reagent was added in 1ml of reaction mixture, boiled for 10 minutes, cooled. Changes in absorbance were recorded at 570nm.

Estimation of tissue superoxide dismutase enzyme

Levels of SOD in the cell free supernatant were measured. Briefly 1.3ml of solution A (0.1M EDTA containing

50mM Na2CO3, pH 10.0), 0.5ml of solution B (90µmNBTnitro blue tetra zolium dye) and 0.1ml of solution C (0.6%Triton X-100 in solution A), 0.1ml of solution D (20 mM Hydroxyl amine hydrochloride, pH 6.0) were mixed and the rate of NBT reduction was recorded for one minute at 560nm. 0.1ml of the supernatant was added to the last minute at 560nm. 0.1ml of the supernatant was added to the test cuvette as well as reference cuvette, which do not contain solution D. Finally, the percentage inhibition in the rate of reduction of NBT was recorded as described above. One enzyme unit was expressed as inverse of the amount of protein (mg) require in one minute.

STATISTICAL ANALYSIS

Results are presented as mean ±SD. Statistical significance was performed by One Way ANOVA using SPSS (version 22) to determine the difference among control and test values. Statistical probability of *P<0.05 was considered significant.

RESULTS

Effect of thioacetamide and Coffee treatment on body weight in treated rats

Significant reduction in body weights were observed after 12 weeks thioacetamide administration in test group and test+supp group as compare to control group. However, Test+Supp group significantly regained their body weights after coffee supplementation in phase II. In Supp group, coffee administration increased body weight as compared to Control group whereas control group gained body weight and they received no treatment throughout the study (fig. 1).

Effect of thioacetamide and coffee treatment on liver weight and relative body weight in treated rats

A significant increase in liver weight and relative liver weight was found in Test group after 12 weeks Thioacetamide administration as compared to control group (table 1) while in Test + Supp group, the coffee administration reduced liver weight as well as relative liver weight significantly as compared to test group, however in coffee treated group, the coffee administration reduced liver weight and relative liver weight as compared to control group (P>0.05).

Effect of thioacetamide and Coffee treatment on total and direct bilirubin in treated rats

A chronic thioacetamide administration resulted in increased level of total bilirubin (5.317±0.618*) in test group where as it was significantly reduced in test+ supp group (4.500±1.183*) and remained near to normal in supp group (3.17±0.349*) as compare to control group (table 2).

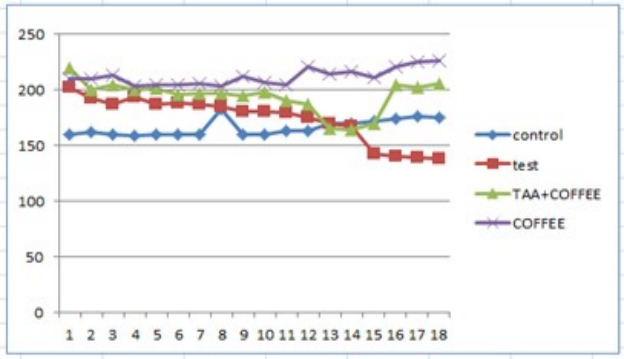

Fig. 1: Effect of thioacetamide and coffee treatment on body weights in treated rats

Level of direct bilirubin (1.420±0.463*) was also significantly increased in test group, Supplementation with coffee markedly reduced it in test + supp (0.812±0.096*) group and was near to normal in supp group (0.6400±0.374*) as compare to control group (table 2).

Effect of thioacetamide and Coffee treatment on serum ALT in treated rats

Significantly increased level of ALT (535±25*) was found in test group where as supplementation with coffee resulted in marked reduction of serum ALT level in test+supp group (280±12.748*) while it remained unchanged in alone supplement group (256.667±5.086) as compare to control group (table-2).

Effect of Thioacetamide and Coffee Treatment on serum ALP in treated rats

Serum ALP level was reduced (223.6±40.153*) in Test group and Test + Supp group (314±25.836*) showed significant increase after coffee supplementation in phase II whereas in supplement group it was also markedly reduced (217.5±16.355*) as compare to control group (table 2).

Effect of Thioacetamide and Coffee Treatment on serum AST in treated rats

Twelve week administration of thioacetamide also resulted in elevated AST level (195.000±33.541*) in test group which was markedly reduced in test + supp group (141±49.8*) and supp group (127.333±13.292*) as compare to control group (table 2).

Effect of thioacetamide and Coffee treatment on superoxide dismutase activity in treated rats.

Table 3 showed a significant reduction in tissue SOD activity in Test group (77±2.290*) as compare to control group. In Test+Supp group (90.372±3.316*), the Coffee administration in second phase restored the SOD activity significantly as compare to control. In supp group (95.140 ±0.302*), the coffee administration caused slightly reduction in SOD activity however almost normal to the control group SOD activity (P<0.05).

Table 1: Effect of thioacetamide and coffee treatment on liver weight and relative body weight in treated rats:

Groups	Liver Weights	Relative Weights
Control	6.750±0.524	0.024±0.001
Test	8.400±0.822*	0.035±0.009*
Test+Supplement	7.800±0.0.274*	0.029±0.017*
Supplement	5.800±1.037*	0.019±0.009*

Table 2: Effect of thioacetamide and coffee treatment on total and direct bilirubin and Serum ALT, AST, ALP activities in treated rats.

Parameters	Control	TEST	Test+Supplement	Supplement
Total bilirubin(unit/L)	3.833±1.366	5.317±0.618*	4.500±1.183*	3.17±0.349*
Direct bilirubin(unit/L)	0.650±0.217	1.420±0.463*	0.812±0.096*	0.6400±0.374
Alanine-amino transferase (unit/L)	257.3±25	535±25*	280±12.748*	256.667±5.086
Aspartates–amino transferase (unit/L)	157.333±5.428	195.000±33.541*	141 ±49.8*	127.333±13.292*
Alkaline phosphatase unit/L)	328±56.331	223.6±40.153*	314±25.836*	217.5±16.355*

Table 3: Effect of thioacetamide and coffee treatment on superoxide dismutase, malondialdehyde and catalase activity in treated rats.

Parameters	Control	Test	Test+supplement	Supplement
Superoxide dismutase unit/gm of tissue.	97.073±0.687	77±2.290*	90.372±3.316*	95.140±0.302*
Malondialdehyde nmol/gm of tissue.	93.500±2.387	114.476±5.348*	103.290±1.133*	94.350±3.609
Catalase nmol/gm of tissue.	11.712±2.214	28.344±11.794*	15.930±1.088*	13.025±1.862*

Values are Mean ± Standard Deviation. Significant differences between Control, Test, Test + Supp, Supp groups by SPSS One-Way ANOVA. P<0.05.

Effect of thioacetamide and coffee treatment on MDA level in treated rats

Table 3 showed that tissue MDA level was significantly increased in Test group (114.476±5.348*) as compare to control. In second phase of Test+Supp group (103.290 ±1.133*), the coffee administration significantly reduced the tissue MDA level as compared to test group whereas in Supp group (94.350±3.609*), the tissue MDA level was almost normal as compared to control (P>0.05).

Effect of thioacetamide and coffee treatment on catalase activity in treated rats

Table 3 showed a significant increase in tissue Catalase activity in Test group (28.344±11.794*) as compare to control. However in Test+Supp group (15.930 ±1.088*), Coffee administration in second phase reduced the tissue Catalase enzyme activity significantly as compare to control while in Supp group (13.025 ±1.862*), catalase enzyme activity was slightly increased however almost normal as compares to control (P>0.05).

DISCUSSION

Liver, the largest organ in the body, carried out many vital functions such as transamination. deamination, dis-intoxication, conversion of ammonia in urea, carbohydrate, lipid, protein metabolism, fatty acid oxidation and several other mechanisms are performed by liver (Johnson, 1995; Nelson and Cox, 2000). The exogenous compounds are majorly metabolized in liver. The end products of these compounds are highly toxic and immediately cause hepatic damage or initiate liver ailments (Ishak *et al*., 1991). Liver comprised of many polyunsaturated fatty acids which are more susceptive to damage by reactive species via oxidative stress (Tawfik and Al-badr, 2012).

Thioacetamide induced liver encephalopathy and toxicity in rodents is highly established to investigate liver preventive characteristics. Thioacetamide metabolically altered via liver cytochrome P450 system into Thioacetamide-S-oxide. The Thioacetamide-S-oxide caused alteration in permeability of cells, enhanced calcium concentration within cells, increase size of nucleus; expand nucleoli, and suppressed mitochondrial mechanisms and this ultimately caused necrosis (Neal and Halpert, 1982). Thioacetamide decreased the number of hepatocytes and amount of oxygen uptake. The biliary cells and damaged hepatocytes caused bile acid to cumulate within the liver; this moreover prompts hepatic injury (Isabel, 2009). Thioacetamide treatment to rats exerted remarkable loss in body weight because of reduction in skeletal muscles and adipose tissues (Devlin, 1997). Elevated liver weight and liver to body weight ratio were observed in Thioacetamide treated rats. Assessing liver to body weight ratio (relative liver weight) is very specific to evaluate alteration in size of liver comparatively with the assessment of liver weight only because liver weight mainly relied on rats' size. The increased size of liver in Thioacetamide treated rats is an

indication of liver damage related to the Thioacetamide harmful effects. These liver weight modulations might be the deposition of collagen and extracellular matrix protein inside liver (Pinzani and Roumbout, 2004).

AST, ALT activities in serum are well-known indicator of liver function while their elevated levels are correspondent to liver damage (Zhao *et al*., 2015; Kadasa *et al*., 2015). ALT, AST activities are thought as the very particular indicator of hepatic damage. They are mainly located in hepatic cell cytoplasm therefore the hepatic injured cells enhanced cellular penetration and cause immediate release of these enzymes into circulation (Ezz *et al*., 2015; Ramaiah, 2007). ALP formed in bile canalcular cells, it increased in a condition of blockage or inflammation of biliary ducts. Reduced ALP levels are due to low magnesium level because ALP activity is prevented by zinc or magnesium chelation (they are enzyme cofactors) (Baldi *et al*., 1993). Increased Total Bilirubin is due to less absorption or less conjugation by liver due to hepatic cell abnormal function whereas raised level of direct or conjugated bilirubin is the result of reduced elimination from liver or maybe the blockage of bile ducts (Sanjiv, 2002).

There is a recommendation about liver damaging drugs inclusive of TAA prompted hepatic damage through producing free radicals, and these radicals later bound with cell's lipids consequently enhance lipid peroxidation (Fadhel and Amran, 2002). The finding of the present study (elevated MDA level in TAA group) also supports this recommendation (Saad *et al*., 2013). (Lee, 2000) described that caffeine removes Hydroxyl radicals formed from Fenton reaction (Fe^{+2}/ H_2 O_2). Moreover, he documented that caffeine in minute concentration reduced microsomal lipid peroxidation in liver through inhibition (Lee, 2000). The reduced SOD activity specified continuous formation of free radicals and this resultantly caused cell deterioration (Abu *et al*., 2005). SOD is a key enzyme to clean up superoxide radicals whereas catalase enzyme metabolized this H_2O_2 (Abraham and Singh, 1999).

Coffee worldwide is the most usual drink up refreshment. Caffeine is the key ingredient in coffee exhibited wide array of activities in living organisms. Moreover, coffee also comprised of chlorogenic acid which possess antioxidative, antimutagenic activity and also the antagonists of hypertensive, hypercholesterolemia and anti-inflammatory properties (Sugiyama *et al*., 1999). It comprises of various different elements like quinides, Trigonelline, lignan, carbohydrate, lipids, nitrogenous compounds, vitamin, minerals, alkaloids, phenolic compounds, melanoidins and 800 volatile compounds (Butt and Sultan, 2011). Coffee reduced the Tumor growth factorβ1 protein expression which could be related to the decrease in collagen accumulation and fibrosis. Gressner *et al*., documented about caffeine which powerfully lowers the expression of Tumor growth factorβ caused connective tissue growth factor in hepatocytes through activating (tumor growth factor β effector) and up-regulation of peroxisome proliferator-activated receptor gamma (PPAR-γ)(Gressner *et al*., 2008; Chakraborti *et al*., 2003). Caffeine has the capability to alter main enzymes of liver microsomal system, particularly stimulation of CYP1A2 in human beings whereas CYP2C in mice/rats has documented. Consequently, coffee has the capability to interrupt in metabolism of hepatic damaging drugs or exogenous compounds and alter equilibrium among stimulation and elimination of these compounds (Huber *et al*., 2008). Coffee ingredients promote enzyme formation and secretion. It has also documented that consuming coffee is also related to the low development of long term hepatopathies. Lipids in coffee also caused modulation in ALT and GGT. The diterpenes and lipids in coffee are also susceptive in reducing liver enzymes. cafestol, kehweol components in coffee take part in carcinogenic detoxification. Furthermore, cafestol and kehweol have documented that they not only increase function of phase II enzyme however also increase hepatic glutathione activity (Cavin *et al*., 1998; Majer *et al*., 2005; Cavin *et al*., 2002; Huber *et al*., 2002; Huber *et al*., 2002). Our results also showed that coffee administration ameliorates liver markers as well as antioxidant enzymes in Thioacetamide induced liver cirrhosis. Montella *et al*., carried out research on coffee correspondent to liver carcinoma. He recommended from his research that coffee has a dose dependent effect; the coffee consumption in adequate quantity gives positive effects in liver cancer (Montella *et al*., 2007; Tanaka *et al*., 1998).

CONCLUSION

It can be concluded that coffee successfully attenuates thioacetamide induced liver cirrhosis.

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